DEVELOPMENT OF ANALYTICAL METHODS FOR THE SIMULTANEOUS ESTIMATION OF LAFUTIDINE AND DOMPERIDONE IN TABLET DOSAGE FORM

Dissertation Submitted to

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Chennai - 600032

In partial fulfillment for the award of the Degree of

MASTER OF PHARMACY
(Pharmaceutical Analysis)

Submitted by

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(Accredited By "NAAC" with CGPA of 2.74 on a Four point Scale at "B" grade)

MELMARUVATHUR – 603319

MAY – 2012

CERTIFICATE

This is to certify that the research work entitled **"DEVELOPMENT OF ANALYTICAL METHODS FOR THE SIMULTANEOUS ESTIMATION OF LAFUTIDINE AND DOMPERIDONE IN TABLET DOSAGE FORM"** submitted to The Tamil Nadu Dr. M.G.R Medical University in partial fulfillment for the award of the Degree of Master of Pharmacy (Pharmaceutical Analysis) was carried out by **GARLAPATI VAMSI KRISHNA (Register No: 26106123)** in the Department of Pharmaceutical Analysis under my direct guidance and supervision during the academic year 2011-2012.

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Date:

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GARLAPATI VAMSI KRISHNA

DEDICATED TO

MY FAMILY

&

FRIENDS

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LIST OF ABBREVIATIONS USED

ICH	-	International Conference on Harmonization
λ	-	Lambda
LOD	-	Limit of Detection
LOQ	-	Limit of Quantification
µg/ ml	-	Microgram Per Milliliter
mg/ tab	-	Milligram Per tablet
mL	-	Millilitre
mM	-	Milli Mole
nm	-	Nanometer
pH	-	Negative Logarithm of Hydrogen Ion Concentration
%	-	Percentage
% RSD	-	Percentage Relative Standard Deviation
RP-HPLC	-	Reverse Phase - High Performance Liquid Chromatography
Rt or t_R	-	Retention Time
S.D.	-	Standard Deviation
S.E	-	Standard Error
UV-VIS	-	Ultraviolet - Visible
IR	-	Infra Red
AUC	-	Area Under Curve
$^{\circ}C$	-	Degree Celsius
Gms	-	Grams

µl	-	Microlitre
rpm	-	Rotations Per Minute
µ	-	Micron
v/v	-	Volume/Volume
min	-	Minute
mL/min	-	Milliliter/Minute
LAF	-	Lafutidine
DOM	-	Domperidone
I.m	-	Intra muscular

INTRODUCTION

INTRODUCTION

1. PHARMACEUTICAL ANALYSIS

(Sethi *et al*., 2003, Gurdeep R. Chatwal *et al.*, 2007)

Pharmaceutical Analysis comprises those procedures necessary to determine the identity, strength, quality and purity of pharmaceuticals.

It got two branches**:**

Quality Control

Quality Assurance

Quality control

It is primarily designed to detect and correct defects or checking to demonstrate whether the anticipated results are complied with.

Quality assurance

It is oriented towards preventing defects from occurring. It is a managerial function which prevents problems by heading them off and by advising restraints and redirection at the proper time and level.

1.1 ANALYTICAL CHEMISTRY (Anjaneyulu *et al.*, 2006)

A scientific discipline that develops and applies methods, instrument and strategies to obtain information on the composition and nature of matter in space and time.

1.1.1 BRANCHES OF ANALYTICAL CHEMISTRY

Biology

Geology

Chemistry

Environmental Science

Material Science

Social science

Medicine

Engineering

Physics

Agriculture

1.1.2 DISCIPLINES OF ANALYTICAL CHEMISTRY

There are two types of analysis. Those are as follows:

Qualitative analysis

Quantitative analysis

1.1.2.1 Qualitative analysis

This is practiced in order to establish the composition of naturally occur, artificially synthesized or manufactured substances.

1.1.2.2 Quantitative analysis

There are several methods for quantitative analysis such methods are as mentioned below.

1.1.3 Chemical methods

1.1.4 Titrimetric analysis

The titrimetric methods are considered superior to gravimetric methods the analysis is similarly based on equivalent weight of one substance reacts quantitatively with the other.

i. Acid-base titrations
ii. Non-aqueous titrations
iii. Redox titrations
iv. Precipitation titrations
v. Complexometric titrations

1.1.5 Gravimetric analysis

The method involves the conversion of the element or a radical to be determined into a pure stable compound, readily convertible into a form suitable for weighing.

1.1.6 Gasometric analysis

The gases like Cyclopropane, Carbondioxide, Nitrous oxide, Oxygen, Octyl nitrate, Nitrogen, Amylnitrate, Ethylene and Helium are determined by Gasometric analysis. The measurement of volume of gas is usually done by means of gas buretts (or) nitrometers.

1.1.7 Physicochemical methods

These methods are mainly used for the measurement of certain physical properties and to determine the contents or composition of a substance. They are employed for the determination of trace concentrations of elements in preference to the sample. These are preferred due to their selectivity, simplicity, speed of analysis and accuracy than other methods. Most of the methods make use of standards having in the known amount of the constituents serves as the basis of comparison in the instrument. The changes in the properties of system detected by measurement of current, potential, electrical conductivity, specific rotation and refractive index.

1.1.8 Microbiological methods

Many micro organisms produce within themselves in chemical substances, which when excreted, interfere with the growth or metabolism of micro organisms. Such compounds are known as antibiotics and need to be present in low concentrations to bring out the antibiotic action. Antibiotics are Chemotherapeutic agents. In micro biological methods comparison of the inhibition of the growth of the bacteria by a measured concentration of antibiotic which is to be examined is

compared with the known concentration of the antibiotic standard preparation having known activity. Widely used methods are cup plate method and tube assay method.

1.1.9 Biological methods

When the potency of the drug or its derivative cannot be properly determined by physical or chemical methods, and where it is possible to observe the biological effects of the drug on some type of living matter, the biological assays are carried out on the basis of such assays is to determine how much of sample gives the same biological effect as a given quantity of the standard preparation. The sample and standard tested under identical conditions in all respect. In a typical bio-assay, a stimulus is applied to a subject. The intensity of stimulus applied to a subject is referred to as the dose and is indicated by a weight or in terms of the concentration of the preparation. The application of a stimulus on a subject produces some observable effect and this is called the response. The response may be measured by the total weight or weight of some organ of the subject, blood sugar concentration, diameter of inhibition zone or by some other physiological symptoms.

1.2 Factors Affecting the Choice of Analytical Methods (Mendham *et al.,* 2002)

Analytical techniques have different techniques of sophistication, sensitivity, selectivity requires different cost and time. Analyst should select best procedure for a given determination.

- ❖ The form of chemical analysis required.
- ❖ Difficulty arising from the nature of the substance
- ❖ Feasible interference from apparatus of the material other than those of interest.
- ❖ The concentration range which needs to be examined.
- ❖ The precision required.

- ❖ The amenities available.
- ❖ The time necessary for total analysis.
- ❖ Analogous type of investigation performed.

1.3 ANALYTICAL METHOD BASED ON MEASUREMENT OF PROPERTY

(Gurdeep R. Chatwaal *et al.,* 2007)

S.No	**Physical property**	**Analytical methods based on measurement of property**
1.	Mass	Gravimetric
2.	Volume	Volumetric
3.	Electrical potential Electrical resistance Electrical current Electrical charge	Potentiometry, Chronopotentiometry. Conductometry. Polarography, Amperometry. Columetry.
4.	Absorption of radiation	Spectrophotometry
5.	Emission of radiation	Emission spectroscopy, flame photometry, fluorescence, radio chemical methods
6.	Scattering of radiation	Turbidimetry, Nephelometry, Raman spectroscopy
7.	Refraction of radiation	Refractometry, Interferometry
8.	Rotation of radiation	Polarimetry, Optical rotatory dispersion
9.	Diffraction of radiation	X-ray electron diffraction methods
10.	Mass to charge ratio	Mass spectrometry
11.	Thermal properties	Thermal conductivity and Enthalpy methods

1.4 APPLICATIONS OF ANALYTICAL CHEMISTRY

(Anjaneyulu *et al.,* 2006)

1. Helping to save lives
2. Consumer and Enviroment protection
3. Farming support
4. Crime prevention and detection
5. Maintain fair play

1.5 UV-SPECTROSCOPY (Gurdeep R. Chatwal *et al.,* 2007)

Ultraviolet absorption spectra arise from transition of electrons within a molecule or an ion from a lower to higher electronic energy levels and the ultraviolet emission spectra arise from the reverse type of transition. For radiation to cause electronic excitation, it must be in the UV region of the electromagnetic spectrum.

Ultraviolet region produces changes in the electronic energy of the molecule resulting from transitions of valence electrons in the molecule. Three different types of electrons are involved in organic molecules. They are follows,

I. σ - electrons

These electrons are involved in saturated bonds, such as those between carbons and hydrogen in paraffins.

II. π - electrons

These electrons are involved in unsaturated hydrocarbons. Typical compounds with π bonds are trienes and aromatic compounds.

III. n - electrons

These are the electrons which are not involved in the bonding between atoms in molecules. Examples are organic compounds containing nitrogen, oxygen and halogens.

1.5.1 Electronic transitions (Sharma, *2007)*

A molecule is excited by the absorption of energy (UV or Visible light). Its electrons are promoted from a bonding to an anti bonding orbital.

i. The antibonding orbital which is associated with the excitation of σ electrons is called σ* anti bonding orbital. So σ ⟶ σ* transition take place when σ (sigma) electron is promoted to antibonding (σ*) orbital. It is represented as σ ⟶ σ* transition.

ii. When a non-bonding electron gets promoted to an antibonding sigma orbital (σ*) then it represents n ⟶ σ* transition.

iii. Similarly π ⟶ π* transition represents the promotion of π electrons to an antibonding π* orbital.

iv. When an n- electron (non- bonding) is promoted to anti bonding π* orbital. It represents π ⟶ π* transition.

1.5.2 Electronic excitation energies

a) σ ⟶ σ* transition

The organic compounds in which all the valence shell electrons are involved in the formation of sigma bonds do not show absorption in the normal ultraviolet region, but occur in vacuum UV region (125-135 nm).

E.g. Methane, Ethane, Propane, Cyclopropane.

b) n ⟶ σ* transition

This type of transition takes place in saturated compounds containing one hetero atom with unshared pair of electrons (n electrons).

E.g. Alcohol, Ethers, Amines, etc.

Such transition require comparatively less energy than that required for σ ⟶ σ* transitions.

c) $\pi \longrightarrow \pi^*$ transition

This type of transition occurs in the unsaturated centers of the molecule in compounds containing double or triple bonds and also in aromatics. The excitation of π electrons requires smaller energy and hence, transition of this type occurs at longer wavelength.

E.g. Alkenes, Alkynes, Aromatic compounds, etc.

d) $n \longrightarrow \pi^*$ transition

In this type of transition an electron of unshared electron pair on hetero atom gets excited to π^* antibonding orbital. This type of transition requires least amount of energy out of all the transitions.

1.5.3 Laws of absorption (Gurdeep R. Chatwal *et al.*, 2007)

There are two fundamental laws related to the absorption of monochromatic radiant energy by homogeneous transparent systems. These are Beer's Law and Lambert's Law.

1.5.4 Beer's law

The intensity of a beam of monochromatic light decreases exponentially with the increase in concentration of the absorbing substance arithmetically.

$I = I_0 e^{-kc}$

Where, I_0 = intensity of incident light

I = Intensity of emerged light

1.5.5 Lambert's law

When a beam of light of light is allowed to pass through a transparent medium, the rate of decrease in the intensity of transmitted light with the thickness of medium is directly proportional to the intensity of the incident light.

$$\frac{-dI}{dt} \propto I$$

From these laws, the following empirical expression – Beer and Lambert's Law

$A = \epsilon ct$

Where, A= Absorbance or optical density or extinction co-efficient

ϵ = Molecular extinction co-efficient

c = Concentration of drug

t = Path length

1.5.6 Limitations of Beer - Lambert's law (Sharma, 2007)

1. When different forms of the absorbing molecules are in equilibrium as in keto-enol tautomers.
2. When fluorescence compounds are present.
3. When solute and solvent forms complex through some sorts of association.

1.5.7 Deviations from Beer's law (Gurdeep R. Chatwal *et al.,* 2007)

From Beer's law it follows that if we plot absorbance against concentration, a straight line passing through the origin should be obtained. But there is usually a deviation from a linear relationship between concentration and absorbance and an apparent failure of Beer's law may ensure. Deviations from the law are reported as positive or negative according to whether the resultant curve is concave upwards or concave downwards.

Deviations from Beer's law can arise due to the following factors:

I. Beer's law will hold over a wide range of concentration provided the structure of the colored ion or of the colored non-electrolyte in the dissolved state does not change with concentration. If a colored solution is having a foreign substance whose ions do not react chemically with the colored components, its small concentration does not affect the light absorption, may

affect light absorption and may also alter the value of the extinction coefficient.

II. Deviations may also occur due to the presence of impurities that fluoresce or absorb at the absorption wavelength. This interference introduces an error in the measurement of absorption of radiation penetrating the sample.

III. Deviations may occur if monochromatic light is not used.

IV. Deviations may occur if the width of slit is not proper and, therefore, it allows undesirable radiations to fall on the detector. These desirable radiations might be absorbed by impurities present in the sample which would cause an apparent change in the absorbance of the sample.

V. Beer's law cannot be applied to suspensions but the latter can be estimated calorimetrically after preparing a reference curve with known concentration.

1.6 Choice of Solvent (Gurudeep R Chatwal *et al.*, 2007)

Solvent used of ultraviolet spectroscopy should posses the following activities

- Solvent under region of investigation should not absorb radiation
- Polarity of solvent should not influence solute molecules.
- High purity of solvent should be maintained.

1.6.1 List of Common Solvents Used in UV-Spectroscopy (Willam Kemp, 1996)

S. No.	SOLVENT	CUT OFF WAVELENGTH (nm)
1	Water	190
2	Ethanol	205
3	Methanol	210

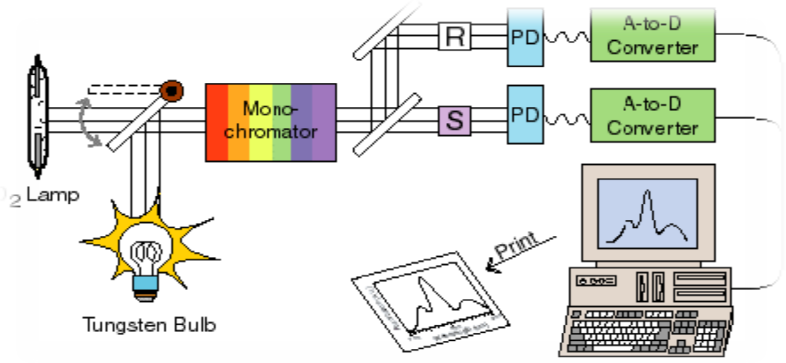
4	Hexane	210
5	Cyclohexane	210
6	Diethyl ether	220
7	Chloroform	245
8	Carbon tetra chloride	265
9	DMF	240
10	Acetone	330

1.6.2 Solvent effects :

The absorption maximum is also influenced by the nature of solvent

1. If absorbing substance is non polar, the effect of solvent is mainly determined by the refractive index of solvent
2. The dipole movement of the solvent is mainly responsible for the change in the position of absorption band
3. The diene systems are however independent of the solvent polarity
4. The absorption maximum for polar compounds is usually shifted with the change in the polarity of solvent.

1.7 Instrumentation



(Anonymous.www.upchurch.com.cn/images/TechInfo/hplcThumb.gif)

The following are the components of a typical UV–Visible spectrophotometer,

a) Radiation source
b) Monochromator
c) Sample holder
d) Detector
e) Signal processor and readout

1.8 Quantitative analysis (Beckett and Stenlake, 2007)

The assay of an absorbing substance may be quickly carried out by preparing a solution in a transparent solvent and measuring its absorbance at a suitable wavelength. The wavelength normally selected is a wavelength of maximum absorption (λ_{max}), where small errors in setting the wavelength scale have little effects on the measured absorbance.

a. Assay of substances in single component samples

Quantitative analysis (assay of an absorbing substance) can be done using following methods.

i. Use of a standard absorptivity values

This method can be used for estimation of drug from formulations or raw material, when reference standard not available. The use of standard A (1 %, 1 cm) value avoids the need to prepare a standard solution of the reference substance in order to determine its absorptivity, and is of advantage in situations where it is difficult or expensive to obtain a sample of the reference substance.

ii. Use of calibration graph

In this procedure the absorbances of a number (typically 4-6) of standard solutions of the reference substance at concentrations encompassing the sample concentrations are measured and a calibration graph is constructed. The concentration

of the analyte in the sample solution is read from the graph as the concentration corresponding to the absorbance of the solution. Calibration data are essential if the absorbance has a non-linear relationship with concentration, or if the absorbance or linearity is dependent on the assay conditions. In certain visible spectrophotometric assays of colorless substances, based upon conversion to colored derivatives by heating the substance with one or more reagents, slight variation of assay conditions, e.g. pH, temperature and time of heating, may rise to a significant variation of absorbance, and experimentally derived calibration data are required for each set of samples.

iii. Single or double point standardization

The single point procedure involves the measurement of the absorbance of a sample solution and of a standard solution of the reference substance. The standard and the sample solution are prepared in similar manner; ideally the concentration of the standard solution should be close to that of the sample solution. The concentration of the substance in the sample is calculated using following formula.

$$\mathbf{C_{test} = A_{test} \times C_{std} / A_{std}}$$

Where,

C_{test} and C_{std} are the concentration in the sample and standard solutions respectively.

A_{test} and A_{std} are the absorbance of the sample and standard solutions respectively.

In double point standardization, the concentration of one of the standard solution is greater than that of the sample while the other standard solution has a lower concentration than the sample. The concentration of the substance in the sample solution is given by

$$C_{test} = \frac{(A_{test} - A_{std1})(C_{std1} - C_{std2}) + C_{std1}(A_{std1} - A_{std2})}{A_{std1} - A_{std2}}$$

Where,

C_{std} is the concentration of the standard solution.

A_{test} and A_{std} are the absorbance of the sample and standard solution respectively. Std_1 and std_2 are the more concentrated standard and less concentrated standard respectively.

b. Assay of substances in multi component samples

The spectrophotometric assay of drugs rarely involves the measurement of absorbance of samples containing only one absorbing component. The pharmaceutical analyst frequently encounters the situation where the concentration of one or more substances is required in samples known to contain other absorbing substances which potentially interfere in the assay. Unwanted absorption from these sources is termed irrelevant absorption and if not removed, imparts systematic errors to the assay of the drug in the sample. A number of modifications to the simple spectrophotometric procedure for single-component samples are available to the analyst, which may eliminate certain sources of interferences and permit the accurate determination of one or all of the absorbing components.

The basis of all the spectrophotometric technique for multicomponent samples is the property that at all wavelengths:

a) The absorbance of a solution is the sum of absorbances of the individual components.

b) The measured absorbance is the difference between the total absorbance of the solution in the sample cell and that of the solution in the reference (blank) cell.

The determination of the multi-component samples can be done by using the following methods,

i. Simultaneous equations method.

ii. Absorbance ratio method (Q-Analysis).

iii. Geometric correction method.

iv. Orthogonal polynomial method.

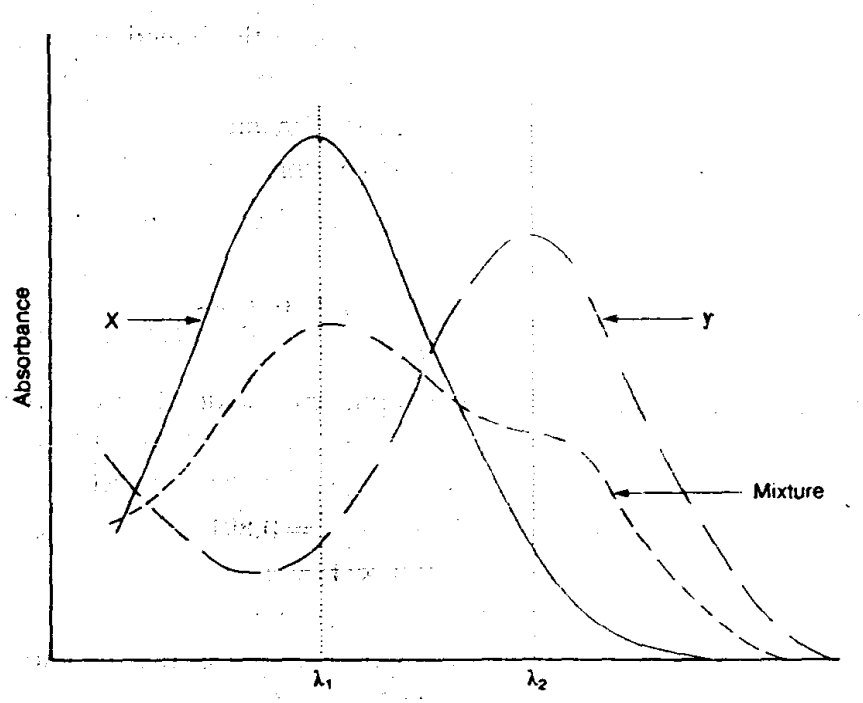
v. Difference spectrophotometry.

vi. Derivative spectrophotometry.

vii. Chemical derivatisation.

i. Simultaneous equation method

If a sample contains two, absorbing drugs (X and Y) each of which absorbs at the λ_{max} of the other as shown in Fig, it may be possible to determine both drugs by the technique of simultaneous equations (Vierodt's method).



The Information required is:

a) The absorptivities of X at λ_1 and λ_2, ax_1 and ax_2, respectively

b) The absorptivities of Y at λ_1 and λ_2, a_{y_1} and a_{y_2}, respectively

c) The absorbance of the diluted sample at λ_1 and λ_2, A_1 and A_2 respectively.

Let Cx and Cy, be the concentrations of X and Y respectively in the diluted sample.

Two equations are constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of the individual absorbance's of x and y,

At λ_1 $\quad \mathbf{A_1 = a_{x_1}bcx + a_{y_1}bcy} \quad$ ---------- (1)

At λ_2 $\quad \mathbf{A_2 = a_{x_2}bcx + a_{y_2}bcy} \quad$ ---------- (2)

For measurement in 1cm cells, b = 1.

Rearrange eq. (2)

$$\mathbf{Cy = \frac{A_2 - a_{x_2}cx}{a_{y_2}}}$$

Substituting for Cy in eq, (1) and rearranging gives

$$\mathbf{Cx = \frac{A_2a_{y_1} - A_1a_{y_2}}{a_{x_2}a_{y_1} - a_{x_1}a_{y_2}}} \qquad (3)$$

and

$$\mathbf{Cy = \frac{A_1a_{x_2} - A_2a_{x_1}}{a_{x_2}a_{y_1} - a_{x_1}a_{y_2}}} \qquad (4)$$

As an exercise we should derive modified equations containing a symbol (b) for path length, for application in situations where A_1, and A_2 are measured in cells other than 1 cm path length.

$$\mathbf{\frac{A_2/A_1}{a_{x_2}/a_{x_1}}} \quad \text{and} \quad \mathbf{\frac{a_{y_2}/a_{y_1}}{A_2/A_1}}$$

Criteria for obtaining maximum precision, based upon absorbance ratios, have been suggested (Glenn, 1960) that place limits on the relative concentrations of the components of the mixture. The criteria are that the ratios should lie outside the range 0.1-2.0 for the precise determination of Y and X respectively. These criteria are

satisfied only when the λ_{max} of the two components are reasonably dissimilar. An additional criterion is that the two components do not interact chemically, thereby negating the initial assumption that the total absorbance is the sum of the individual absorbance. The additivity of the absorbance should always be confirmed in the development of a new application of this technique. The British Pharmacopoeia assay of quinine-related alkaloids and cinchonine-related alkaloids in cinchona bark is based upon this technique.

ii. Absorbance ratio method

The absorbance ratio method is a modification of the simultaneous equations procedure. It depends on the property that, for a substance which obeys Beer's law at all wavelengths, the ratio of absorbances at any two wavelengths is a constant value independent of concentration or pathlength. For example, two different dilutions of the same substance give the same absorbance ratio A_1/A_2, 2.0. In the USP, this ratio is referred to as a Q value. The British Pharmacopoeia also uses a ratio of absorbances at specified wavelengths in certain confirmatory tests of identity. For example, Cyanocobalamin exhibits three λ_{max} at 278 nm, 361 nm and 550 nm. The A_{361}/A_{550} is required to be 3.30 ± 0.15 and the A_{361}/A_{278} to be 1.79 ± 0.09.

iii. Geometric correction method

A number of mathematical correction procedures have been developed which reduce or eliminate the background irrelevant absorption that may be present in samples of biological origin. The simplest of these procedures is the three-point geometric procedure, which may be applied if the irrelevant absorption is linear at the three wavelengths selected.

iv. Orthogonal polynomial method

The technique of orthogonal polynomials is another mathematical correction procedure which involves more complex calculations than the three – point correction procedure. The basis of the method is that an absorption spectrum may be represented in terms of orthogonal functions as follows:

$$\mathbf{A(\lambda) = p_0 P_0(\lambda) + p_1 P_1(\lambda) + p_2 P_2(\lambda) \ldots p_n P_n(\lambda)}$$

Where A denotes the absorbance at wavelength λ belonging to a set of n + 1 equally spaced wavelengths at which the orthogonal polynomials, $P_0(\lambda)$, $P_1(\lambda)$, $P_2(\lambda) \ldots P_n(\lambda)$ are each defined.

v. Area under the curve method

From the spectra obtained for calculating the simultaneous equation, the area under the curve were selected at a particular wavelength range for both the drugs were each drug have its absorption. The "X" values of the drugs were determined at the selected AUC range. The "X" value is the ratio of area under the curve at the selected wavelength range with the concentration of the component in mg/ml. These "X" values were the mean of six independent determinations. A set of two simultaneous equations were obtained by using mean "X" values. And further calculations are carried out to obtain the concentration of each drug present in the sample.

vi. Derivative spectroscopic method

This method involves the conversion of the normal spectrum into first, second or higher derivative spectrum. The transformation that occurs in the derivative spectrum is understood by reference to a Gaussian band which represents an ideal absorption band.

The first derivative (D^1) spectra is a plot of the ratio of change of absorbance with wavelength against wavelength, i.e., a plot of slope of the fundamental spectrum

against wavelength or a plot of dA/ dλ Vs λ_1. At λ_2 and λ_4, the maximum positive and maximum negative slope respectively in the D°. Spectrums correspond with maximum and minimum respectively in the D^1 spectrum. The λ_{max} at λ_3 is a wavelength of zero slope and gives dA/d λ_0, i.e., a cross-over point, in the D1 spectrum .

The first order derivative spectrum of absorption band is characterized by a maximum, a minimum and a cross-over at a λ_{max} of the absorption band. These spectral transformations confer two main advantages on derivative spectrophotometry. Firstly an even order spectrum is of narrower spectral band width than its fundamental spectrum. Derivative spectrum shows better resolution of overlapping bands than the fundamental spectrum and may permit the accurate determination of λ_{max} of the individual bands. Secondly, derivative spectroscopy discriminates in favors of the substances of narrow spectral bandwidth against broad band width substances.

1.9 INTRODUCTION TO HPLC METHODS OF ANALYSIS OF DRUGS IN COMBINED DOSAGE FORM (Gurudeep R Chatwal *et al.*, 2008)

High performance liquid chromatography [HPLC] was developed in the late 1960's and 1970's it is widely accepted separation technique for both sample analysis and purification in a variety of areas including the pharmaceutical, biotechnological, environmental polymer and food industries.

HPLC instrumentation is made up of eight basic components they are mobile phase reservoir, solvent delivery system, sample introduction device, column, detector, waste reservoir, connective tubing and a computer, integrator (or) recorder.

Chromatography is defined as a method of separating a mixture of components into individual components through equilibrium distribution between two phases. Chromatography technique is based on the difference in the rate at which the

components of a mixture move through a porous medium (stationary phase) under the influence of some solvent or gas (mobile phase).

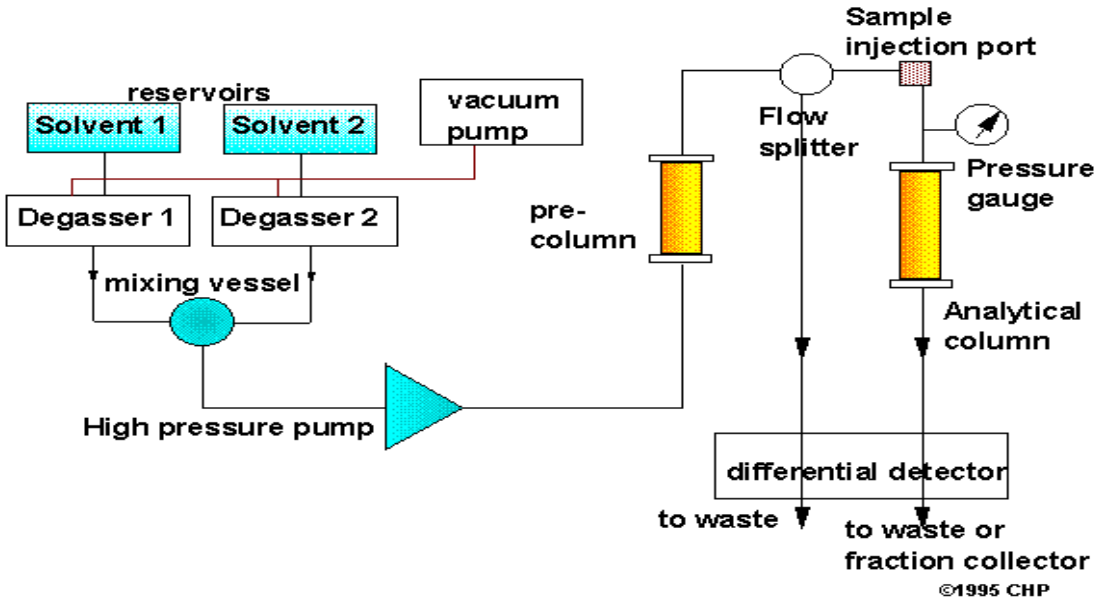
The chromatographic method of a separation in general involves the following steps:

- Adsorption or retention of a substance or substance on the stationary phase.
- Separation of the adsorbed substance by the mobile phase.
- Recovery of the separated substance by a continuous flow of the mobile phase. The method being called elution.
- Quantitative and qualitative analysis of the eluted substance

1.9.1 Introduction to HPLC (Sharma, 2006)

HPLC is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC instrument consists of four basic parts

- The column
- Detector
- Injection system
- Mobile-phase pump system



A schematic diagram of HPLC equipment

1.9.2 Principle of separation in HPLC (Willard et al., 1986)

The principle of separation in normal phase and reverse phase mode is the adsorption. When a mixture of components is introduced in to a HPLC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent travels slower. The components which have less affinity towards the stationary phase travel faster. Since no two components have the same affinity towards the stationary phase the components are separated.

1.9.3 Modes of chromatography

i. Normal phase mode

ii. Reverse phase mode

i. Normal phase chromatography

In normal phase mode, the stationary phase (silica gel) is polar in nature and the mobile phase is non-polar. In this technique non-polar compounds travel faster and eluted first. The silica structure is saturated with silicon groups at the end and 'OH' groups attached to silicon atoms are the active binding sites.

ii. Reverse phase chromatography

In reverse phase technique, a non polar stationary phase is used. The mobile phase is polar in nature hence polar components get eluted first and non-polar compounds are retained for a longer time. Since most of the drugs and pharmaceutical are polar in nature, they are not retained for a longer and eluted faster, which is advantageous. Different columns used are ODS (Octa DecylSilane) or C_{18}, C_8 and C_4 etc.

1.9.4 Types of pumps

- Reciprocating Piston Pumps

- Syringe Type Pump
- Constant Pressure Pump

1.9.5 Columns for HPLC (Willard *et al*., 1986)

The columns most commonly used are made from precision bore polished stainless steel tubing; typical dimensions are 10-30 cm long and 4 (or) 5 mm internal diameter. The stationary phase (or) packing is retained at each end by thin stainless steel frits with a mesh of 2 µm or less. The packing used in modern HPLC consist of small, rigid particles having a narrow particles size distribution. The types of column used in HPLC are

- Standard columns
- Radial compression columns
- Narrow Bore columns
- Short, fast columns
- Guard columns and In-line filters

1.9.6 Detectors **(**Ashutoshkar, 2005)

The main function of the detector in HPLC is to monitor the mobile phase coming out the column, which in turn emits electrical signals that are directly proportional to the characteristics either of the solute or the mobile phase.

Basic detector requirements

An ideal LC detector should have the following properties:

1. Low drift and noise level (particularly crucial in trace analysis).
2. High sensitivity.
3. Fast response.
4. Wide linear dynamic range (this simplifies quantitation).
5. Low dead volume (minimal peak broadening).

6. Cell design which eliminates remixing of the separated bands.
7. Insensitivity to changes in type of solvent, flow rate, and temperature.
8. Operational simplicity and reliability.
9. It should be tunable so that detection can be optimized for different compounds.

On-Line Detectors

1. Refractive index.
2. UV/Vis Fixed wavelength.
3. UV/Vis Variable wavelength.
4. UV/Vis Diode array.
5. Fluorescence.
6. Conductivity.
7. Mass-spectrometric (LC/ MS).

Off-Line Detector

1. FTIR spiral disk monitor.

1.10 VALIDATION

(www.askaboutvalidation.com/forum/showthread.php?t=1175)

The word "Validation" means "Assessment" of validity or action of proving effectiveness.

Types of validation

The validation is divided into different types. They are,

Prospective validation

This method is employed when historical data of the Product is not available or is not sufficient and in process and finished product testing is not adequate to ensure reproducibility or high degree of compliance to product likely attributes.

Retrospective validation

This provides trend of comparative result (i.e.) review and evaluation of existing information for comparison when historical data is sufficient and readily available.

Concurrent validation

Based on information generated during implementation of a system for this extensive testing and monitoring are performed as part of initial run of the method.

Re-validation

Revalidation provides the evidence that changes in a process and are the process environment, introduced either intentionally or unintentionally, do not adversely affect process characteristic and product quality.

There are two basic categories of revalidation. Revalidation in case of known change (including transfer of process from one company to another or from one site to another) Periodic revalidation carried out at scheduled intervals.

Reasons/ purpose of validation (Sethi, 2001)

i. Setting standards of evaluation procedures.
ii. Taking appropriate action in case of non-compliance.
iii. Retrospective validation is useful for trend comparison of results compliance to cGMP/ cGLP.
iv. Closer interaction with pharmacopoeia forum to address analytical problems. Enables scientist to communicate scientifically and effectively on technical matters.

1.11 ANALYTICAL METHOD VALIDATION

(Code Q2A;Q2B. ICH Guidelines1994 and 1996)

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Methods need to be

validated or revalidated. The International Conference of Harmonization (ICH) of technical requirements for the registration of pharmaceutical for human use has developed a consensus text on validation of analytical procedures. The document includes definition for eight validation characteristics.

The parameters as defined by the ICH and by other organizations

- ✓ Specificity
- ✓ Selectivity
- ✓ Precision
    - Repeatability
    - Intermediate precision
    - Reproducibility
- ✓ Accuracy
- ✓ Linearity
- ✓ Range
- ✓ Limit of detection
- ✓ Limit of quantification
- ✓ Robustness
- ✓ Ruggedness

SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to present. An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and assay.

ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or on an accepted reference value and the value found.

Assay

Assay of Active substances

Assay of Medicinal products

Several methods are available to determine the accuracy

a) Application of an analytical procedure to an analyte of known purity
b) Comparision of the results of the proposed analytical procedure
c) Application of the analytical procedure to synthetic mixtures

Impurity (Quantification)

Accuracy should be assessed on sample spiked with known amounts of impurities. It should be clear how the individual or total impurities are to be determined.

Eg: weight/weight or area percent

PRECISION

The precision of an analytical procedure expresses the closeness of the agreement between a series of measurements obtained from multiple sampling of same homogeneous sample under the prescribed conditions. Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.

Repeatability (intra- assay precision)

Express the precision under small operating conditions over a short interval of time. It should be assessed using a minimum of nine determinations.

Intermediate Precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. Typical validation to be studied includes days, analysts, equipments etc.

Reproducibility

Reproducibility is assessed by means of an inter-laboratory trail. Reproducibility should be considered in case of the standardization of an analytical procedure, for insistence inclusion of procedure in pharmacopoeias.

LINEARITY

Linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte sample.

RANGE

Range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample including these concentrations for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

LIMIT OF DETECTION

The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected.

a. Based on visual evaluation
b. Based on Signal-to-Noise ratio
c. Based on the standard deviation of the response and the slope
    - Based on the standard deviation of blank
    - Based on the calibration graph

LIMIT OF QUANTIFICATION

The quantification limit is generally determined by the analysis of samples with the known concentrations of analyte and by establishing the minimum value at which the analyte can be quantified with acceptable accuracy and precision

a. Based on visual evaluation

b. Based on Signal-to- Noise ratio

c. Based on the standard deviation of the response and the slope

- Based on the standard deviation of blank
- Based on the calibration graph

ROBUSTNESS

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It shows the reliability of an analysis with respect to deliberate variations in the method parameters.

RUGGEDNESS

The united states of pharmacopoeia (USP) define ruggedness as the degree of reproducibility of test results obtained by the analysis of the same sample under a variety of normal test condition such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst.

1.12 SYSTEM SUITABILITY PARAMETERS (Anonymous USP, 1995; Sethi 2001)

System suitability test are an integral part of gas and liquid chromatography. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. These tests are based on the concept

that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such. FDA guidelines on "Validation of chromatographic methods" the following acceptance limits are proposed as initial criteria.

1) Capacity Factor (or) Retention (K_A)

The retention of a drug with a given packing material and eluent can be expressed as retention time or retention volume, but both of these are dependent on flow rate, column length and column diameter. The retention is best described as a column capacity ratio (K), which is independent of these factors. The column capacity ratio of a compound (A) is given as

$$K_A = \frac{V_A - V_0}{V_0} = \frac{t_A - t_0}{t_0}$$

2) Resolution (R_S)

The resolution, R_s of two neighboring peaks is defined by the ratio of the distance between the two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of R_s is 2.0. It is calculated by using the formula,

$$R_f = \frac{Rt_2 - Rt_1}{0.5\,(W_1 + W_2)}$$

Where,

Rt_1 and Rt_2 are the retention times of components 1 and 2

W_1 and W_2 are peak widths of components 1 and 2

3) Selectivity (α)

The selectivity (or separation factor) α, is a measure of relative retention of two components in a mixture. The ideal value of selectivity is 2. It can be calculated by using the formula,

$$\alpha = \frac{V_2 - V_0}{V_1 - V_0}$$

Where, V_0 is the void volume of the column and V_2 and V_1 are the retention volumes of the second and the first peak, respectively.

4) Column efficiency

Efficiency, (N) of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Smaller the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 5,000 to 100,000 plates/ meter are ideal for a good system. Efficiency is calculated by using the formula,

$$N = 16 \frac{Rt^2}{W^2}$$

Where, R_t is the retention time and W is the peak width.

5) Peak asymmetry factor (A_s)

Peak asymmetry factor, A_s can be used as a criterion of column performance. The peak half width b of a peak at 10 % of the peak height, divided by the corresponding front half width a gives the asymmetry factor.

1.4 PHARMACEUTICAL STATISTICS

Linear regression

Linear regression is a statistical technique that defines the functional relationship between two variables by best-fitting straight line. Once a linear relationship has been shown to have a high probability by the value of the correlation coefficient ‘r’, then the best straight line through the data points has to be estimated. This can often be done by visual inspection of the calibration graph, but in many cases it is far more sensible to evaluate the best straight line by linear regression (the method of least squares)

The equation of straight line is

$$y = mx + c$$

Where, y the dependent variable is plotted as result of changing x, the independent variable.

To obtain the regression line 'y on x' the slope 'm' of the line and the intercept 'c' on the y axis are given by the following equation.

$$m = \frac{N \Sigma xy - (\Sigma x)(\Sigma y)}{N \Sigma x^2 - \Sigma(x)^2}$$

And

$$C = \frac{(\Sigma y)(\Sigma x^2) - (\Sigma x)(\Sigma y)}{N \Sigma x^2 - \Sigma(x)^2}$$

Correlation coefficient (r)

It is a procedure commonly used to characterize quantitatively the relationship between variable. Correlation is related to linear regression. To establish whether there is a linear relationship between two variables x_1 and y_1**,** use Pearson's correlation coefficient 'r'.

$$r = \frac{n\Sigma x_1 y_1 - \Sigma x_1 y_1}{\{[n\Sigma x_1^2 - (\Sigma x_1)^2][n\Sigma y_1^2 - (\Sigma y_1)^2]\}^{1/2}}$$

Where n is the number of data points.

The value of 'r' must lie between +1 and -1, the nearer it is to +1, the greater the probability that a definite linear relationship exists between the variables x and y, values close to +1 indicate positive correlation and values close to -1 indicate negative correlation values of 'r' that tend towards zero indicate that x and y are not linearly related (they made be related in a non-linear fashion).

Standard deviation (SD)

It is commonly used in statistics as a measure of precision statistics and is more meaningful than is the average deviation. It may be thought of as a root-mean-square deviation of values from their average and is expressed mathematically as

Where,

$$S = \sqrt{\frac{\sum_{i=1}^{i=n} (x_i - \bar{x})_2}{N-1}}$$

S is standard deviation.

If N is large (50 or more) then of course it is immaterial whether the term in the denomination is N -1 or N

Σ = sum

$\bar{x}$ = Mean or arithmetic average.

$x - \bar{x}$ = deviation of a value from the mean

N = Number of observations

Percentage relative standard deviation (% RSD)

It is also known as coefficient of variation (CV). It is defined as the standard deviation (S.D) expressed as the percentage of mean.

$$CV \text{ or } \% \text{ RSD} = \frac{S.D}{\bar{x}} \times 100$$

Where,

S.D is the standard deviation,

$\bar{x}$ = Mean or arithmetic average.

The variance is defined as S^2 and is more important in statistics than S itself. However, the latter is much more commonly used with chemical data.

Standard error of mean (SE)

Standard error of mean can be defined as the value obtained by division of standard deviation by square root of number of observations. It is mathematically expressed as

$$S.E. = \frac{S.D.}{\sqrt{n}}$$

Where,

S.D = Standard deviation = number of observations.

Literature Review

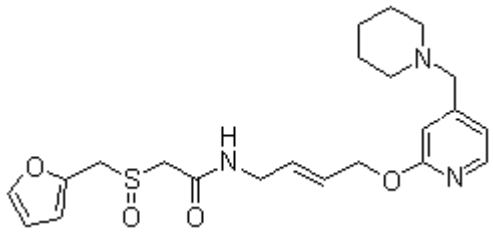
2. LITERATURE REVIEW

2.1 DRUG PROFILE

(TheMerck Index 2006; http://www.rxlist.com/script/main/srchcont_ rxlist.asp?)

LAFUTIDINE

Molecular structure



Chemical name

2-[(2-furylmethyl)sulphinyl]-N-((2Z)-4-{[4-(piperidin-1-ylmethyl)pyridin-2-yl]oxy}but-2-en-1-yl)acetamide.

Molecular formula

$C_{22}H_{29}N_3O_4$

Molecular weight

431.55 g/mol

Category

H_2- Receptor antagonist, Anti- Secretory agent.

Description

An off-white or white crystalline powder.

Solubility

It is Freely soluble in DMF, Glacial Acetic Acid, soluble in Methanol, sparingly soluble in dehydrated ethanol.

Identification

1) Melting Point

Standard Value	Observed Average Value*
92.7- 94.9°C	92.5°C

*Average of five observations

2) IR Spectrum

IR Spectrum of Lafutidine and interpreted and shown in the figure 1.

Mechanism of Action

Lafutidine an H_2- Receptor antagonist inhibits gastric acid secretion not only in nights but also day time.

Pharmacokinetics

a) Distribution: Lafutidine is 88% bound to plasma proteins, mostly albumin.

b) Metabolism: Metabolised by CYP3A4(mainly), CYP2D6.

Contraindications

Lactation.

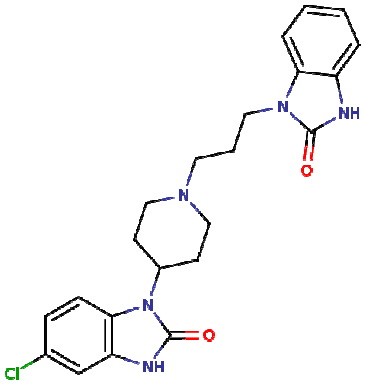
Drug Interactions and precaution

Constipation, hyperuricaemia, increase in total bilirubin, gynaecomastia, anorexia, hallucinations.

2.2 DOMPERIDONE

(The Merck Index 2006; http://www.rxlist.com/script/main/srchcont)

Molecular structure



Chemical name

5-chloro-1-[1-[3-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)propyl]-piperidin-4-yl]-1,3-dihydro-2H benzimidazole-2-one.

Molecular formula

$C_{22}H_{24}ClN_5O_2$

Molecular weight

425.91 g/mol

Category

Dopamine antagonist

Description

White or almost white crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in alcohol and methanol, soluble in DMF.

Identification

1) Melting Point

Standard Value	Observed Average Value*
236 - 239°C	238.8°C

*Average of five observations

2) IR Spectrum

IR Spectrum of Domperidone is compared with the standard values and principal peaks at a wave numbers were identified. IR spectrum is shown in the figure 2.

Storage Protected from light.

Mechanism of Action and pharmacology

Domperidone stimulates gastric muscle contraction by antagonizing the inhibitory effect of dopamine on post synaptic cholinergic neurons in the myenteric plexus. Dopamine effectively increases esophageal peristalisis and lower esophageal sphincter pressure, increases gastric motility and peristalisis, enhances gastroduodenal coordination and consequently facilitates gastric emptying.

Pharmacokinetics

a) Absorption

Peak plasma levels of Domperidone occur within 10-30 min after i.m injection and 30 min after oral administration.

b) Metabolism

Hydroxylation and Oxidative N-dealkylation

Adverse effects

Domperidone elevates serum prolactin levels, galactorrhoea, gynaecomastia, menstrual irregularities, thirst, nausea and diarrhoea.

2.3 REPORTED METHODS

2.3.1 FOR LAFUTIDINE :

1. **Wei- Dong Chen** *et al.* (2006), Simple, sensitive and rapid LC-ESI-MS method for the quantification of Lafutidine in human plasma- Application to pharmacokinetic studies Lafutidine and internal standard were isolated from plasma samples by liquid-liquid extraction with diethyl ether using column C_{18} (150mm* 2.0mm, 5µm) at the flow rate of 0.2mL\min and with gradient elution using electro spray ionization detection source.

2. **Lili Wu** *et al.* (2009), Determination of Lafutidine in human plasma by high performance liquid chromatography – electro spray ionization mass spectrometry : Application to bio – equivalence study, Determined the concentration of Lafutidine in human plasma by adding diazepam as internal standard and 1M NaoH solution to 0.5 ml of plasma sample, Lafutidine was extracted from plasma with Hexane : Isopropanol (95 : 5 % v\v) using v p ODS C_{18} (250 * 2.0 mm) column with mobile phase methanol : water (20mm CH3COONH4) in the ratio 80 : 20 % v/v and electro spray ionization interface and SIM mode detection.

3. **YE Xiang- fa** *et al.* (2003), Determination of Lafutidine and its tablets by high performance liquid chromatography using ODS C_{18} column with mobile phase Methanol : 0.2M Ammonium acetate solution : Triehylamine in the ratio 70 : 30: 0.1 % v/v/v and the detection wavelength was set at 275 nm.

4. **Ding Hong-liang** *et al.* (2009), Determination of Lafutidine by HPLC using C_{18} column with mobile phase Methanol : 0.1M Sodium dihydrogen phosphate solution in the ratio 40 : 60 v/v with detection wave length set at 279 nm.

5. **Shangguan Ying – ying**, *et al.* (2005), Determination of related substances in Lafutidine by RP-HPLC using C_{18} (4.6mm * 150mm, 5µm) column with mobile

phase Methanol : Acetonitrile : 0.02M Phosphate buffer in the ratio 25 : 15 : 60 v/v/v adjusting the P^H to 6.0 with Triethylamine at the flow rate 1 mL/min and the detection wavelength was set at 220 nm.

6. **Onodera** *et al.* (1999), Gastro protective mechanism of Lafutidine, a novel anti ulcer drug with histamine H2 – receptor antagonist activity. Investigated the gastro protective activity of Lafutidine by examining the effect on ammonia induced change in trans- mucosal potential difference (PD), basal gastric mucosal blood flow (GMBF) and noxious agent induced cell damage.

7. **Shen Qian** *et al.* (2007), Determination of Lafutidine concentration in human blood serum by HPLC using supercosil TM LC- 18-DB (4.6mm *250, 5µm) column with mobile phase Methanol : Sodium Acetate buffer in the ratio 1:2 at P^H 4.5 using fluorescence detector at excitation wavelength at 285 nm and emission wavelength at 313 nm.

8. **Zheng Heng** *et al.* (2008), Determination of Lafutidine in human plasma by HPLC-MS. Determined by taking 10µl of Ranolazine HCl (2.0 µg/mL internal standard) was added to 0.5 ml of plasma. The plasma samples extracted with 5ml ether. The residue was dissolved in carbinol, 2 µl was injected in to ODS column with mobile phase as carbinol and the flow rate was set at 0.2 mL/min.

9. **Shimatani** *et al.* (2006), Lafutidine, a newly developed anti-ulcer, elevates post prandial intragastric P^H and increases plasma Calcitonin gene related peptide and Somatostatin concentrations in humans : Comparison with Famotidine.

10. **Sumitra** *et al.* (2011), Reported Analytical method development and validation of Lafutidine in tablet dosage form by RP-HPLC, Determined using Hypersil silica C_{18} (250 * 4.6, 5µ) column with mobile phase 0.02M Dihydrogen Potassium Orthophosphate and Acetonitrile in the ratio 30: 70 at a flow rate of 1 mL/min.

11. **Zhen Yong** *et al*. (2006), Reported determination of Lafutidine in serum with RP-HPLC, Determined by using c_{18} column with mobile phase Methanol and 0.005M Ammonium Dihydrogen phosphate at a flow rate of 1 mL/min, set a detection wavelength of 218 nm.

12. **Kouzuro Ikawa** et al. Pharmacokinetic and pharmacodynamic properties of Lafutidine after post prandial oral administration in healthy subjects: Comparison with famotidine.

2.3.2 FOR DOMPERIDONE

1. **Lakshmana Prabu** *et al,* (2008), Simultaneous estimation of Esomeprazole and Domperidone by U.V Spectrophotometric method, estimated by using simultaneous eqution method using methanol as solvent the wavelengths selected are 301 nm and 284 nm.

2. **Priti D. trivedi** *et al.* (2010), Estimation of Esomeprazole and Domperidone by Absorption ratio method in pharmaceutical dosage form, estimated using methanol as solvent, Isobestic point was set at 290 nm.

3. **Ramesh Sawanth** *et al.* (2010), Validated Spectrophotometric methods for simultaneous estimation of Paracetamol, Domperidone and Tramadol HCl in pure and tablet dosage form, Determined by using 0.1N NaoH. **Method 1:** Three wavelength spectrophotometry, the absorbances of sample solution was measured at 256 nm, 289.6 nm, for the estimation of Paracetamol and Domperidone respectively and Tramadol HCl does not absorb at the wavelengths. Estimated of Tramadol HCl is carried out at 218.4 nm. **Method 2**: Based on multiwavelength spectroscopic method.

4. **Ravikumar** *et al.* (2006), Simultaneous estimation of Domperidone and Pantaprozole in solid dosage form by U.v spectrophotometry, Determined by

simultaneous equation method using cramels rule and Absorption ratio method based on measurement absorptivity at 216, 287 and 290 nm.

5. **Patel** *et al.* (2010), Development and validation of Derivative spectrophotometric method for simultaneous estimation of Domperidone and Rabeprazole Sodium in bulk and dosage form, determined by using first derivative spectrophotometry at wavelengths selected at 253 , 266.4 nm in sodium hydroxide solution.

6. **Sohan S. chitlange** *et al.* (2010), Simultaneous spectrophotometric estimation of DexRabeprazole and Domperidone in capsule dosage form, Determined by using simultaneous equation at 258.5 and 286.5 nm wavelengths and multicomponent mode of analysis at 258 and 286.5 nm and area under curve at 263.5-253.5 and 291.5-281.5 nm using methanolic HCl (0.1) as solvent.

7. **Karla Kapil** *et al.* (2009), Spectrophotometric method for Simultaneous estimation of Paracetamol and Domperidone in tablet dosage formulation, Developed by simultaneous equation method with wavelengths selected at 250, 285 nm with methanol as solvent.

8. **Venkatesh** *et al.* **(**2010), Spectrophotometric method for simultaneous estimation of Paracetamol and Domperidone in tablet dosage form, Domperidone and Paracetamol were estimated by Simultaneous equation method at 284.5 nm and 244.5 nm using 1N Glacial Acetic acid as solvent.

9. **Koujirou Yamamoto** *et al.* (1988), Quantitative determination of Domperidone in rat plasma by HPLC with fluorescence detection, Determination of Domperidone in rat plasma involves Liquid- Liquid extraction followed by RP-HPLC with fluorometric detection at 282 nm for excitation and 328 nm for emission.

10. **Lakshmi Sivasubramanian** *et al.* (2007), Simultaneous HPLC estmation of Omeprazole and Domperidone from tablets, Estimated by using Hypersil ODS C_{18}

(150 * 4.6mm, 5µ) column with mobile phase methanol : 0.1M Ammonium acetate (PH 9) in the ratio 60 : 40 v/v at a flow rate of 1 mL/min and the detection wavelength was set at 280 nm.

11. **Shozan Mondal** *et al.* (2011), Development and validation of RP-HPLC method for the simultaneous estimation of Domperidone and Naproxen in tablet dosage form, Determined by using two LC-20 AT pump, SPD- 20A UV detector, SIL- 20A auto sampler and CTO-10 ASVP column oven with C_{18}(250 * 4.6mm, 5µ) column and mobile phase was Phosphate buffer (P^H adjusted to 3 with NaoH) : methanol in the ratio 30 : 70 v/v at a flow rate of 1 mL/min and detection wavelength was set at 280 nm.

12. **Ahsanul Haque** *et al.* (2011), Validated RP-HPLC method for estimation of Ranitidine HCl, Domperidone and naproxen in solid dosage form, Estimated in shim-pack CLC- ODS(250 * 4.6 mm, 5µ) column with mobile phase 0.1M Orthophosphoric acid solution (P^H 3): Methanol in the ratio 35: 65 v/v at a flow rate of 1 mL/min and Uv detection was set at 280 nm.

13. **Sivakumar** *et al.* (2007), Development and validation of a RP-HPLC method for simultaneous determination of Domperidone and Pantoprazole in pharmaceutical dosage forms, Determined by using ODS analytical column with mobile phase as Methanol : Acetonitrile : Triehylamine solution (P^H 7 adjusted with 85% phosphoric acid) in the ratio 20 : 33 : 47 v/v/v, at a flow rate of 1 mL/min and UV detection was set at 285 nm.

14. **Rakesh Kumar Singh** *et al.* (2010), RP-HPLC method development and validation for simultaneous estimation of Ranitidine HCl and Domperidone in combined tablet dosage form, Determined efficient separation on phenomenax C_{18} (250 * 4.6mm, 5µ) column using mobile phase Phosphate : Acetonitrile : Methanol in

the ratio 40 : 30 : 30 v/v/v in an isocratic mode of elution at a flow rate of 1.5 mL/min and detection wavelength was set at 210 nm.

15. **Vijaya Ratna** *et al.* (2011), Reported RP-HPLC Method development and validation of Domperidone Maleate determined by using Luna C_{18} phenomenex (250 * 4.6mm,5µ) column with mobile phase methanol and water in the ratio 50 : 50 v/v, the detection wavelength was set at 285 nm.

16. **Namitha** *et al.* (2006), Quantification of Domperidone, Paracetamol, Esomeprazole and Lansoprazole in pharmaceutical dosage forms by RP-HPLC using superco c_{18} column with a mobile phase 0.01M Dipotassium hydrogen phosphate : Acetonitrile in the ratio 65: 35 v/v at a flow rate of 1 mL/min set at a detection wavelength of 285 nm.

Aim & Plan of Work

3. Aim and plan of work

The combination dosage form selected for the present study contains Lafutidine and Domperidone in solid oral dosage forms, recently this combination has been approved by DCGI (Drug Controller General India)

In the view of the literature cited, method for estimation of Lafutidine and Domperidone individually and in combination with other drugs were reported, but no work is reported in the combination of both the drugs

For UV method,

1. Find the drugs solubility in various solvents
2. To determine maximum absorbance and overlaid the spectrums
3. Determining the standard absorbance for all selected wavelength for each drugs
4. Development of simple, precise, accurate and sensitive
   - Simultaneous equations method
   - Area under curve method
5. Validation of developed methods as per ICH guidelines

For RP-HPLC method,

1. A suitable mobile phase were selected for two drugs with proper resolution and short retention time
2. Development of chromatogram with various concentration for each drug to determine range of concentrations
3. Development of chromatogram in formulation
4. Validation of the developed method

Materials & Methods

4. MATERIALS AND METHODS

4.1. MATERIALS USED

4.1.1. DRUGS

Lafutidine and Domperidone were generously gifted by Orchid Pharmaceuticals Pvt. Ltd., Chennai and the formulation containing Lafutidine 10 mg and Domperidone 30 mg tablets were purchased from a local pharmacy with the brand name LAFAXID- D.

4.1.2. REAGENTS & CHEMICALS

All the chemicals used were of analytical grade and HPLC grade procured from Qualigens, India Ltd. The chemicals used for the study were

Acetonitrile (HPLC grade)

Water (HPLC grade)

Methanol (Analytical grade) and

Ammonium Acetate (Analytical grade)

4.1.3. Instruments Specifications

a) Shimadzu AX – 200 digital balance: (Shimadzu instruction manual)

Specifications	
Weighing capacity	200 gms
Minimum display	0.1 mg
Standard deviation	≤ 0.1 mg
Operation temperature range	5 to 40° C

b) Shimadzu UV – Visible spectrophotometer: (Shimadzu and Elico instruction manuals)

Model : Shimadzu, UV-1700; Double beam UV - Visible spectrophotometer.

ELICO SL – 210; Double beam UV - Visible spectrophotometer.

Specification	Shimadzu UV – 1700	Elico SL – 210
Monochromator	Aberration- correcting concave blazed holographic grating	Concave holographic grating with 1200 lines/ mm
Detector	Silicon photodiode	Photodiode
Stray Light	0.04% or less (220 nm; NAI 10g/ lt) 0.04% or less (340 nm; $NaNo_2$ 50g/ lt).	< 0.05% T at 220 nm with NAI 10g/ lt
Measurement Wavelength range	190 ~ 1100 nm	190 ~1100 nm
Spectral Band width	1 nm or less (190 to 900nm).	1.8 nm
Wave length Accuracy	± 0.5 nm on broad automatic wavelength calibration mechanism.	± 0.5 nm automatic wavelength calibration mechanism.
Recording range	Absorbance; - 3.99 ~ 3.99 Abs Transmittance; - 399 ~ 399%	Absorbance; ± 3.000 Abs
Photometric accuracy	± 0.004 Abs (at 1.0 Abs). ± 0.002 Abs (at 0.5 Abs).	± 0.005 Abs (at 1.0 Abs). ± 0.010 Abs (at 0.5 Abs).
Operating Temperature/ Humidity	Temperature range; 15 to 35˚C Humidity range; 35 to 80% (15 to below 35˚C) 35 to 70% (30 to below 35˚C)	Temperature range; 15 to 35˚C Humidity range; 35 to 80% (15 to below 35˚C) 35 to 70% (30 to below 35˚C)

c) Thermo Scientific Spectra High Performance Liquid Chromatography:

Detector Specifications	
Light source	Deuterium Arc lamp
Measurement wavelength	190 to 700 nm
Spectral Band Width	5 nm
Wavelength Accuracy	± 1 nm
Cell path length	10 nm
Cell volume	20 µl
Operating temperature range	4 to 35° C (39 to 104° F)
Recording range	0.0001 to 4.000 AUFS
Operating temperature/Humidity	4 to 35° C / 75 %

Pump Specifications	
Pump type	Double reciprocating plunger pump
Pumping method	Constant flow delivery and constant pressure delivery
Suction filter	45 µm
Line filter	5 µm mesh
Operating	4 to 35° C (39 to 104° F)

4) Sonica ultra sonic cleaner- model 2200 MH

5) ELICO – pH meter model L1610

6) Micropipette

7) Melting point apparatus - Guna enterprises, Chennai.

4.2. METHODS EMPLOYED

The methods employed for the Simultaneous estimation of Lafutidine and Domperidone are

4.2.1. UV Spectrophotometric method

a) Simultaneous equation method

b) Area under curve method

Selection of solvent

The solubility of drugs was determined in a variety of solvents as per Indian pharmacopoeial standards. Solubility was carried out in non polar to polar solvents. The common solvent was found to be methanol for the analysis of Lafutidine and Domperidone for the proposed method.

Preparation of standard stock solution

10 mg of standard Lafutidine and Domperidone were weighed and transferred into10 mL volumetric flasks separately and dissolved in methanol and made up to the volume with methanol. These solutions were observed to contain 1000 $\mu g\ mL^{-1}$.

Selection of wavelengths for estimation and stability studies

The selection of wavelengths for the estimation Lafutidine and Domperidone a suitable diluted stock solution contain 10 $\mu g\ mL^{-1}$ of each and the solutions were scanned between 200 and 400 nm by using methanol as blank. From the overlaid spectra, by the observation of spectral characteristics of Lafutidine and Domperidone the wavelengths selected were 273 nm and 287.5 nm respectively. The Stability was performed by measuring the absorbance of same solution at different time intervals. It was observed that Lafutidine and Domperidone in methanol were stable for more than 4 hours at the selected wavelengths.

For Area under the curve method, the area under the specified ranges for both the drugs was measured in area calculation mode. Wavelengths selected for Lafutidine is 276-271 nm and 291-282 nm for Domperidone.

Preparation of calibration graph

Further diluted the standard stock solution to contain 80 μg mL^{-1} of Lafutidine and 240 μg mL^{-1} of Domperidone respectively. The aliquots from stock solution of Lafutidine (0.5 – 2. 5 ml of 80 μg mL^{-1}) and Domperidone (0.5–2.5 ml of 240 μg mL^{-1}) were transferred into a 10 mL volumetric flasks and made up to the volume with methanol. The Absorbance of different concentrations were measured at 273 nm and 287.5 nm for Lafutidine and Domperidone. The calibration curve was plotted at their corresponding wavelengths. The two drugs, Lafutidine and Domperidone were linear with the concentration range of 4-20 μg mL^{-1} and 12-60 μg mL^{-1} respectively, at their respective wavelengths to the proposed methods.

Quantification of formulation

Ten tablets of formulation LAFAXID- D (Containing Lafutidine 10 mg and Domperidone 30 mg) were weighed accurately. The average weight of tablets was found and powdered. The tablet powder equivalent to 25 mg of Domperidone was weighed and transferred into a 25 mL volumetric flask, added a minimum quantity of methanol to dissolve the substance by using ultra sonication for 15 minutes and made up to the volume with methanol (1000 μg mL^{-1}). The content was filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 3 mL to 25 mL with methanol in a 25 ml volumetric flask, further dilute 3 mL to 10 mL and obtained 36 μg mL^{-1} of Domperidone and 12 μg mL^{-1} Lafutidine respectively. The absorbance measurements were made 5 times for the

formulation at 273 nm and 287.5 nm. From the absorptivity values of Lafutidine and Domperidone at 273 nm and 287.5 nm, the amount of Lafutidine and Domperidone could be determined by using Simultaneous equation method.

The area measurements were made for the same dilutions in area calculation mode at the selected wavelengths between 276 and 271 nm and 291 and 282 nm. The amount was calculated by using area under curve method.

Recovery studies

The recovery experiment was done by adding known concentrations of Lafutidine and Domperidone raw material to the 50% preanalyzed formulation. Standard Lafutidine and Domperidone in the range of 80%, 100% and 120% is added to the 50% preanalyzed formulation into a series of 10 mL volumetric flasks, and diluted with methanol and the contents were sonicated for 15 minutes then the solution was made up to mark with methanol. After sonication, the solutions were filtered through Whatmann filter paper No. 41. The absorbances of the resulting solutions were measured at their selected wavelengths for the determination of Lafutidine and Domperidone. The amount of each drug recovered from the formulation was calculated by Simultaneous equation method and Area under curve method. The procedure was repeated for three times.

Validation of developed method

Linearity

A calibration curve was plotted between concentration and absorbance. Lafutidine was found linear with the concentration range of 4-20 μg mL^{-1} and Domperidone showed the linearity in the range of 12-60 μg mL^{-1} at selected wavelengths for both the methods.

Accuracy (Recovery studies)

Accuracy of the method was confirmed by recovery studies. To the preanalyzed formulation, a known quantity of raw materials of Lafutidine and Domperidone were added and the procedure was followed as per the analysis of formulation. The amount of each drug recovered was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

Precision

The repeatability of the method was confirmed by the analysis of formulation and repeated for 5 times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The % RSD was calculated. The intermediate precision of the method was confirmed by intraday and inter day analysis, i.e., the analysis of formulation was repeated three times in the same day and on three consecutive days. The amount of drugs was determined and % RSD also calculated.

Ruggedness

Ruggedness of the method was confirmed by the analysis of formulation and performed by the different analysts and with different instruments. The amount and % RSD were calculated.

LOD and LOQ

The linearity study was carried out for six times. The LOD and LOQ were calculated by using the average of slope and standard deviation of intercept.

4.2.3 RP-HPLC METHOD DEVELOPMENT AND OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

Selection of mobile phase and λ_{max}

Solutions of Lafutidine and Domperidone (10 μg mL^{-1}) were prepared in the mobile phase [Methanol : 0.5% Ammonium Acetate Buffer (85:15 v/v)] and scanned in the UV region of 200-400 nm and recorded the spectrums. It was found that the two drugs have marked absorbance at 220 nm and can be effectively applied for estimation of two drugs without interference. Therefore 220 nm was selected as detection wavelength for estimation of two drugs by RP-HPLC method with an Isocratic elution technique.

Stability of sample solutions

Solutions of Lafutidine and Domperidone (10 μg mL^{-1}) absorbance were checked for their stability at 220 nm and it was found that two drugs were stable for approximately three hours.

OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

Initial separation conditions

The following chromatographic conditions were preset initially to get better resolution of Lafutidine and Domperidone.

Mode of operation	-	Isocratic
Stationary phase column	-	Intersil ODS-3V (150 mm × 4.6 mm i.d. 5μ)
Mobile phase	-	Methanol : 0.5% Ammonium acetate buffer
Proportion of mobile phase	-	50 : 50% v/v
Detection wavelength	-	220 nm
Flow rate	-	1 mL/min
Temperature	-	Ambient

Sample load - 20 µL

Operating pressure - 2000 psi

Method - External Standard Calibration method.

The mobile phase was primarily allowed to run for 30 minutes to record a steady baseline. Mixture of Solutions of Lafutidine and Domperidone were injected and the respective chromatogram was recorded. It was found that both Lafutidine and Domperidone were splitted, for this reason different ratios of mobile phase were attempted to obtain good chromatogram with acceptable system suitability parameters.

Selection of mobile phase

A mobile phase contains Methanol and 0.5% Ammonium acetate buffer was selected.

The ratio of the mobile phase is

S. No	Mobile phase	Observation
1	Methanol : 0.5 % Ammonium Acetate Buffer (85:15 v/v)	Both the Lafutidine and Domperidone were eluted with sharp peak

Optimized chromatographic conditions

The following optimized conditions were employed for the analysis of Lafutidine and Domperidone by Isocratic RP-HPLC method.

Mode of operation - Isocratic

Stationary phase column - Intersil ODS-3V (150 mm × 4.6 mm i.d. 5µ)

Mobile phase - Methanol :0.5% Ammonium Acetate buffer

Proportion of mobile phase	-	85 : 15% v/v
Detection wavelength	-	220 nm
Flow rate	-	1 mL/min
Temperature	-	Ambient
Sample load	-	20 µL
Operating pressure	-	2000 psi
Method	-	External Standard Calibration method.

Preparation of standard Lafutidine solution

50 mg of standard Lafutidine was weighed accurately and transferred into a 100 mL volumetric flask and dissolved in methanol, after dissolution the volume was made up to the mark with methanol (500 µg mL^{-1}). Further dilution was made by pipetting 10 mL of standard stock into a 100 mL to acquire 50 µg mL^{-1} solution.

Preparation of standard Domperidone solution

190.89 mg of standard Domperidone equivalent to 150 mg of Domperidone Maleate was weighed accurately and transferred into a 100 mL volumetric flask and dissolved in methanol, after dissolution the volume was made up to the mark with methanol (1500 µg mL^{-1}). Further dilution was made by pipetting 10 mL of standard stock into a 100 mL to acquire 150 µg mL^{-1} solution.

Preparation of Calibration graph

In this progression, the aliquots of stock solution of Lafutidine (0.7 – 1.3 mL of 500 µg mL^{-1}) and Domperidone (0.7 – 1.3 mL of 1500 µg mL^{-1}) were transferred into a 10 ml volumetric flasks and made up to the mark with mobile phase, and the solutions contains 35, 40, 45, 50, 55, 60 and 65 µg mL^{-1} of

Lafutidine and 105, 120, 135, 150, 165, 180 and 195 μg mL^{-1} Domperidone. The solutions were injected and the chromatograms were recorded at 220 nm. The above concentration range was found to be linear and obeys Beer's law. The procedure was repeated for six times. The peak areas were plotted against concentration and the calibration curve was constructed.

Estimation of Lafutidine and Domperidone in tablet formulation

Estimation of Lafutidine and Domperidone in tablet formulation by RP-HPLC was carried out in three levels using optimized chromatographic conditions. Ten tablets of formulation (LAFAXID-D) were weighed accurately. The average weight of tablets was found and powdered. The tablet powder equivalent to 50 mg of Lafutidine was weighed and transferred into a 100 mL volumetric flask and added a minimum quantity of methanol to dissolve the substance and the content was sonicated for 15 minutes, and made up to the volume with methanol then filtered through Whatmann filter paper No. 41.

Assay Procedure

From the above clear solution, the aliquots of 0.8, 1.0, 1.2 mL were taken in to three separate 10 mL flasks and made the volume with mobile phase and obtained 40, 50, 60 μg mL^{-1} solution of Lafutidine and 120, 150, 180 μg mL^{-1} of Domperidone. A steady base line was recorded with optimized chromatographic conditions. After the stabilization of base line for 30 minutes, three test solutions of formulation were injected and recorded the chromatograms. The concentration of each test solution was determined by using slope and intercept values from the calibration graph.

Recovery Experiments

a) Preparation of Lafutidine and Domperidone raw material stock solution

100, 300 mg of Lafutidine and Domperidone were weighed separately and transfer in to a 100 mL standard flask and made up the volume with Methanol and kept aside.

b) Procedure

To each 1 mL of preanalyzed formulation solution (50 μg mL^{-1} of Lafutidine and 150 μg mL^{-1}) added 0.5, 1, 1.5 mL of raw material stock solution into 10mL volumetric flasks and made up to the mark with mobile phase. The procedure was repeated as per analysis of formulation. The quantity of drug recovered was calculated by using slope and intercept values from the calibration graph.

System suitability studies

The system suitability studies conceded as per ICH guidelines and USP. The parameters like capacity factor, tailing factor, asymmetry factor and number of theoretical plate and resolution were calculated.

Mobile Phase

The mobile phase used for RP-HPLC was Methanol : 0.5 % Ammonium Acetate buffer (85 : 15 v/v)

Results & Discussion

5. RESULTS AND DISCUSSION

Estimation of multiple drug formulations have advantage that the methods are less time consuming and usage of solvent is minimized. Two simple, rapid, precise and accurate spectrophotometric methods and an isocratic RP-HPLC method were developed and validated for the estimation of Lafutidine and Domperidone in pure form and in combined tablet dosage form. The raw materials were generously gifted by Orchid Pharmaceuticals Pvt. Ltd. and confirmed by Melting point and I.R spectrum which are shown in Figure 1 and 2. The methods employed are

- UV Spectrophotometric methods

  Simultaneous Equation method

  Area Under Curve method

- RP-HPLC method

5.1. SIMULTANEOUS EQUATION METHOD

The solubility of Lafutidine and Domperidone was determined as per Indian Pharmacopoeia. The numeral polar and non – polar solvents were attempted to dissolve the drugs. From the solubility profile methanol was chosen as a common solvent for the estimation of Lafutidine and Domperidone. The solubility data is in Table 1 for Lafutidine and Domperidone.

The sample solutions of 10 μg mL^{-1} of Lafutidine and Domperidone in methanol prepared individually and the solutions were scanned in UV region in the wavelength range from 200 to 400 nm by using methanol as blank. The individual and overlaid spectra of Lafutidine and Domperidone were recorded as shown in Figure 3, 4 and 5. From the spectrum, 273 nm was chosen as λ_{max} of Lafutidine and 287.5 nm was chosen as λ_{max} of Domperidone, these two wavelengths employed for Simultaneous estimation of Lafutidine and Domperidone respectively. Different

aliquots of Lafutidine in methanol were prepared in the concentration range of 4 -20 μg mL^{-1}. The absorbances of solutions were measured at 273 nm and 287.5 nm. The calibration curve was plotted using concentration against absorbance. The calibration graph at 273 and 287.5 nm is shown in Figure 6 and 7. Different aliquots of Domperidone in methanol were prepared in the concentration range of 12-60 μg mL^{-1}. The absorbances of these solutions were measured at 273 nm and 287.5 nm. The calibration graphs were plotted and are shown in Figure 8 and 9. The preparation of calibration curve was repeated for five times for each drug at their selective wavelengths. The optical parameters like, Sandell's sensitivity, Molar absorptivity, Correlation coefficient, Slope, Intercept, LOD, LOQ and Standard error were calculated. The correlation coefficient for the two drugs was found to be above 0.999. This indicates that all the drugs obey Beer's law in the selected concentration range. Hence the concentrations were found to be linear. The optical characteristics of two drugs at their selective wavelengths are shown in Table 2 for Lafutidine and Table 3 for Domperidone. The formulation LAFAXID-D tablets containing 10 mg of Lafutidine and 30 mg of Domperidone was selected for analysis. The nominal concentration of Domperidone from the linearity (36 μg mL^{-1}) was prepared and also contains (12 μg mL^{-1}) of Lafutidine, the absorbances of the solution were measured at their respective wavelengths. The percentage label claim present in tablet formulation was found to be 99.49 ± 1.874 and 100.36 ± 1.06 for Lafutidine and Domperidone, respectively. The amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be 1.1588 and 0.4664 for Lafutidine and Domperidone, respectively. The low % RSD values indicate that the method has good precision. The results of analysis are shown in Table 4.

Further the precision of the method was confirmed by Intraday and Inter day analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of intraday and inter day analysis were found to be and 0.0510 and 0.8245 for Lafutidine, 0.6245 and 0.6468 for Domperidone. The results of analysis are shown in Table 5. The results showed that the precision of the method was confirmed.

The developed method was validated for Ruggedness. It refers to the specific of one lab to multiple days which may include multiple analysts, multiple instruments and different sources of reagents and so on. In the present work it was confirmed by different analysts and different instruments. The % RSD value by analyst 1 and analyst 2 were found to be 0.9419 and 0.3133 for Lafutidine and 0.2872 and 0.2838 for Domperidone, respectively. The low % RSD values indicate that the developed method was more rugged. The results are shown in Table 6 & 7. The % RSD values by instrument 1 and instrument 2 was found to be 0.9566 and 0.8206 for Lafutidine and 0.4443 and 0.3248 for Domperidone. The results are shown in Table 8 & 9.

The accuracy of the method was performed by recovery studies. To the 50 % preanalysed formulation, a known quantity of Lafutidine and Domperidone raw material solutions were added at different levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 98.125-98.67 % for Lafutidine and 99.32-99.68 % for Domperidone. The % RSD values were 0.2807 and 0.2032 for Lafutidine and Domperidone. These low % RSD value for the two drugs indicates that this method is very accurate. The recovery data is shown in Table 10.

5.2 AREA UNDER THE CURVE METHOD

The solutions containing 10 μg mL^{-1} of Lafutidine and Domperidone in methanol were prepared individually and the solutions were scanned in UV region in the wavelength range from 400-200 nm by using methanol as blank. From the spectrum, 276-271 nm was chosen as the area range for Lafutidine and 291-282 nm was chosen as the area range for Domperidone. This two wave length ranges were used for the estimation of Lafutidine and Domperidone by area under curve method.

Aliquots of Lafutidine and Domperidone in methanol were prepared in the concentration range of 4-20 μg $mL^{-1}$and 12-60 μg mL^{-1}. The area of the solutions was measured at 276-271 nm for Lafutidine and 291-282 nm for Domperidone. The calibration curve was plotted using area against concentration. The calibration curve at 276-271 nm and 291-282 nm for Lafutidine are shown in Figure 10 and 11. The calibration curve of Domperidone at 276-271 and 291-282nm were plotted and are shown in Figure 12 and 13. The preparation of calibration curve was repeated for six times for each drug at their selective wavelengths. The optical parameters like, Sandell's sensitivity, Molar absorptivity, Correlation coefficient, Slope, Intercept, LOD, LOQ and Standard error were calculated. The Correlation coefficient for both the drugs was found to be around 0.999. This indicates that both the drugs obey Beer's law and they were linear at the selected concentration range. The optical characteristics of two drugs at their selective wavelengths are shown in Table 11 & 12. The concentration of solution containing both Lafutidine and Domperidone 36 μg mL^{-1} was prepared and the area of the solutions was measured at their respective wavelength ranges. The percentage purity of formulation LAFAXID-D was found to be 99.49 ± 1.87 and 100.36 ± 1.06 for Lafutidine and Domperidone, respectively. The amount present in formulation was in good concord with the label claim and the

% RSD values were found to be 0.8822 and 0.41407 for Lafutidine and Domperidone, respectively. The results of analysis were shown in Table 13. The low % RSD value indicates that the method was highly precise.

. Further, the precision of the method was confirmed by Intraday and Interday analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of intraday and inter day analysis were found to be 0.8658 and 0.7124 for Lafutidine, 0.2456 and 0.6629 for Domperidone. The results of analysis are shown in Table 14. The results revealed that the method was highly precise.

The developed method was validated for Ruggedness. It refers to the specificity of one lab to multiple days which may include different analysts, different instruments and different sources of reagents and so on. In the present work it was confirmed by different analysts and different instruments. The % RSD value by analyst 1 and analyst 2 was found to be 0.4815 and 0.8316 for Lafutidine and 0.3057 and 1.1126 for Domperidone, respectively. The low % RSD value indicates that the developed method was more rugged. The results were shown in Table 15 & 16. The % RSD values by instrument 1 and instrument 2 was found to be 1.0123 and 0.5407 for Lafutidine and 0.3682 and 0.7037 for Domperidone. The low % RSD value indicates that the developed method was more rugged. The results are shown in Table 17 & 18.

The accuracy of the method was performed by recovery studies. To the 50 % preanalysed formulation, a known quantity of Lafutidine and Domperidone raw material solutions were added at three different concentration levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 98.15-99.19% for Lafutidine and

99.71-100.01% for Domperidone. The average % RSD values of Lafutidine and Domperidone was found to be 0.5541 and 0.1653, respectively. The low % RSD value of drugs indicates that this method was accurate. The recovery data was shown in Table 19.

5.3 RP – HPLC METHOD

An exertion has been made for a simple, rapid, accurate and precise method for the estimation of Lafutidine and Domperidone in pure form and in formulation by an isocratic RP-HPLC method.

The solutions of 10 μg mL^{-1} of Lafutidine and Domperidone in mobile phase [Methanol : 0.5% Ammonium Acetate buffer (85 : 15 % v/v)] were prepared and the solutions were scanned in the range of 200-400 nm. It was found that the two drugs have marked absorbance at 220 nm and can be effectively used for estimation of two drugs without interference. Therefore 220 nm was selected as detection wavelength for the estimation of two drugs by RP-HPLC method with an isocratic elution technique and it was found that the two drugs were stable for approximately one and half hour.

The optimization was done by changing the composition of mobile phase ratio. The mobile phase consists of Methanol: Water (50:50 % v/v) was initially attempted and chromatograms were recorded and shown in Figure 14. Different ratios of mobile phase were attempted and the chromatograms recorded are shown in 15 and 16. Finally the mobile phase consists of Methanol : 0.5% Ammonium Acetate buffer (85:15% v/v). After calculating all system suitability test parameters, Methanol : 0.5% Ammonium Acetate buffer 85 : 15% v/v at a flow rate of 1 mL/min was selected and the optimized chromatograms individually and in combination are shown at Figure 17-19. The retention time of Lafutidine and Domperidone, were found to be

1.287 and 3.342, respectively. The retention time between two drugs indicate that the drugs were separated with better resolution of 2.055. The system suitability parameters for the optimized chromatogram are shown in Table 20.

With the optimized chromatographic conditions, stock solutions of Lafutidine and Domperidone were prepared in mobile phase and prepared the in the concentration range of 35-65 μg mL^{-1} of Lafutidine, 105-195 μg mL^{-1} of Domperidone. 20 μL of each solution was injected and recorded the chromatograms at 220 nm. The chromatograms are shown in Figure 20-26. The calibration curve was plotted using concentration against peak area. The procedure was repeated for three times. The Correlation co-efficient was found to be above 0.999 for the two drugs. The calibration graph of Lafutidine and Domperidone are shown in Figure 27 and 28, respectively. The optical characteristics of Lafutidine and Domperidone are shown in Table 21.

LAFAXID-D formulation containing 10 mg of Lafutidine and 30 mg of Domperidone was selected for analysis. The nominal concentration 150 μg mL^{-1} of Domperidone, which is also contains 50 μg mL^{-1} of Lafutidine in the mobile phase was prepared. 20 μL of each solution was injected and chromatograms were recorded. The percentage purity was found to be 100.42 ± 1.41 and 100.00 ± 0.8684 for Lafutidine and Domperidone, respectively. The precision of the method was confirmed by repeatability of formulation for three times at three levels i.e, 80 %, 100 %, 120 % of the nominal concentration and the chromatograms are shown in Figure 29-37. The % RSD was found to be 0.7093 and 0.2673 for Lafutidine and Domperidone, respectively. It indicates that the method has good precision. The data is shown in Table 22.

The accuracy of the method was performed by recovery studies. To the preanalyzed formulation, a known quantity of Lafutidine and Domperidone raw material solutions were added at different levels, injected the solutions. The chromatograms were recorded as shown in the Figure 38-40. The percentage recovery was found to be in the range between 99.17 and 99.37 % for Lafutidine and 99.35 and 100.81% for Domperidone. The % RSD was found to be 0.9488 and 0.9492 for Lafutidine and Domperidone, respectively. The low % RSD values for recovery indicated that the method was found to be accurate. The values are given in the Table 23.

All the above parameters with the ease of operation ensure that the projected methods could be applied for the routine analysis of Lafutidine and Domperidone in pure form and in tablet dosage forms.

Summary & Conclusion

6. SUMMARY AND CONCLUSION

Simple, rapid, precise and accurate UV- Spectrophotometric method and RP-HPLC method were developed and validated for the estimation of Lafutidine and Domperidone in tablet dosage form.

6.1. UV SPECTROSCOPIC METHODS

From the solubility profile, methanol was chosen as a common solvent for the estimation of Lafutidine and Domperidone. The sample solutions of 10 μg mL^{-1} of Lafutidine and Domperidone in methanol prepared individually and the solutions were scanned in UV region in the wavelength range from 200-400 nm by using methanol as blank. The overlaid spectra of mixture of Lafutidine and Domperidone were recorded. From the spectra, 273 nm for Lafutidine and 287.5 nm for Domperidone was selected as wavelength to construct simultaneous equation.

The percentage label claim present in tablet formulation was found to be 100.00 ± 1.874 and 100.36 ±1.841 for Lafutidine and Domperidone respectively. The percentage recovery was found to be in the range of 97.43-99.37% for Lafutidine and 99.25-100.34% for Domperidone.

6.2 In area under the curve method, the wavelength ranges between 276 and 271 nm and 291 and 282 nm were selected for the estimation of Lafutidine and Domperidone respectively. The percentage label claim present in formulation was found to be 99.49 ± 1.5994 and 100.36 ± 1.0945 for Lafutidine and Domperidone, respectively. The percentage recovery was found to be in the range of 98.15-99.19% for Lafutidine and 99.71-100.01% for Domperidone.

6.3. RP-HPLC METHOD

In RP-HPLC method, mobile phase used is Methanol : 0.5% Ammonium Acetate buffer (85:15% V/V) with flow rate of 1 mL per min, the retention time of

Lafutidine and Domperidone were found to be 1.287 and 3.342, respectively at 220 nm. The retention time of Lafutidine is 1.287 which is less than the acceptable criteria and also the theoretical plate count is less than the acceptable criteria according to ICH guidelines. Because of the time and cost factor included the same.

The percentage purity was found to be 100.26 ± 0.7107 and 100.00 ± 0.4684 for Lafutidine and Domperidone, respectively. The precision of the method was confirmed by repeatability of formulation for three times at three levels. The accuracy of the method was confirmed by recovery studies. The percentage recovery was found to be in the range of 99.17-99.37 % for Lafutidine and 99.35-100.81% for Domperidone. The % RSD was found to be 0.7093 and 0.2673 for Lafutidine and Domperidone, respectively. The low % RSD values for recovery indicated that the method was accurate.

Simple, rapid and accurate UV Spectroscopic (Simultaneous Equation method and Area under curve method) and an isocratic RP-HPLC method showed excellent Sensitivity, Reproducibility, Accuracy, and Repeatability, which is evidenced by low %RSD. The results obtained in recovery studies were indicating that there is no interference from the excipients used in the formulation. By comparing two methods, UV Spectroscopic methods were found to be economic when compared to RP-HPLC. Hence it is suggested that the proposed UV Spectroscopic and an isocratic RP-HPLC methods can be effectively applied for the routine analysis of Lafutidine and Domperidone in bulk and in tablet formulation and the obtained results will be presented elsewhere.

Figures

FIGURE 1

IR SPECTRUM OF LAFUTIDINE

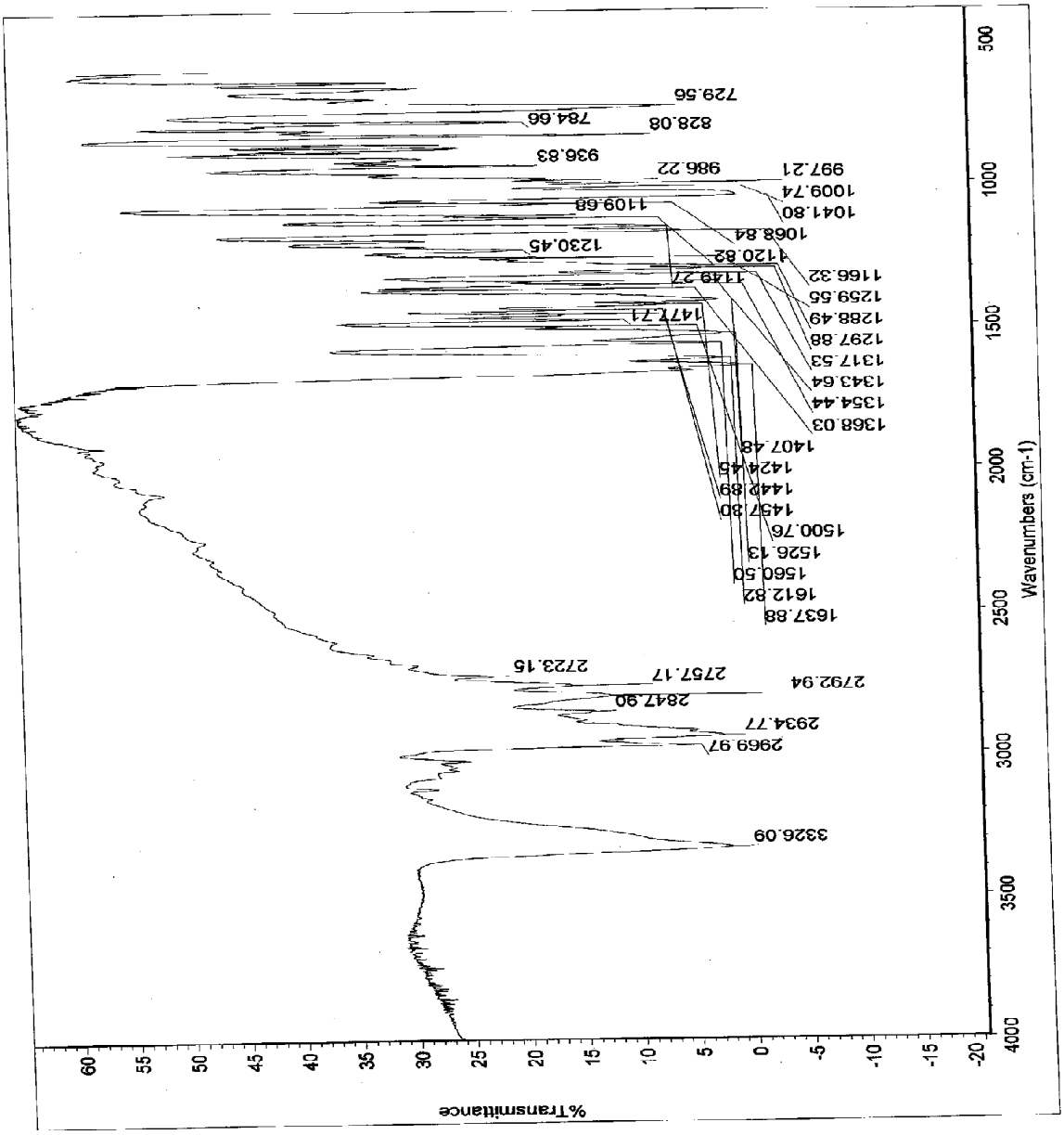


FIGURE 2

IR SPECTRUM OF DOMPERIDONE

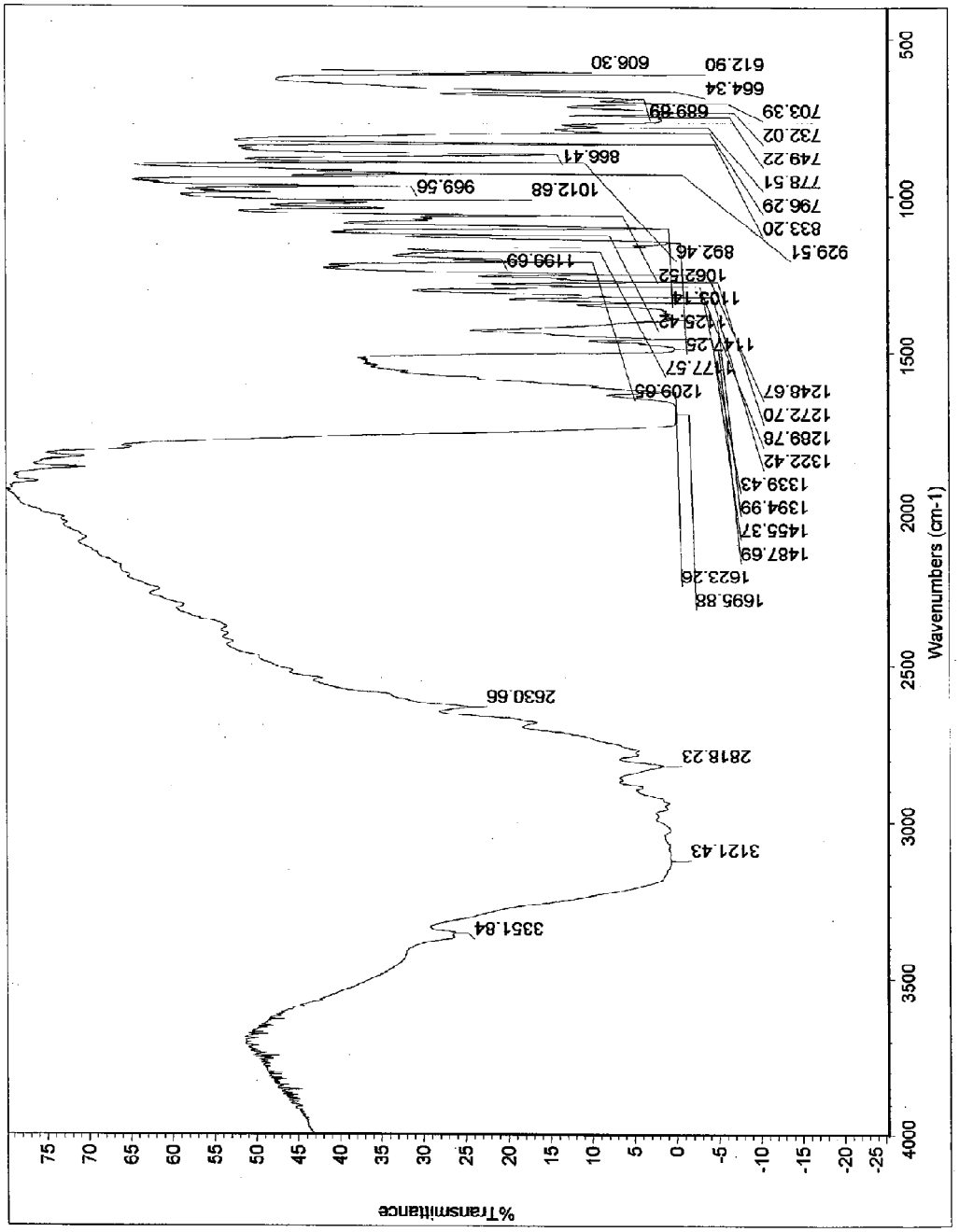


FIGURE 3

UV- SPECTRUM OF LAFUTIDINE IN METHANOL

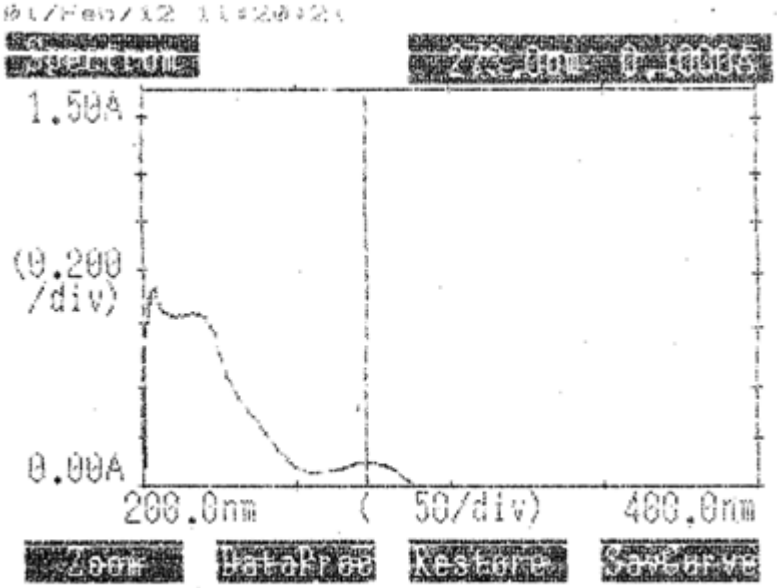


FIGURE-4

UV SPECTRUM OF DOMPERIDONE IN METHANOL

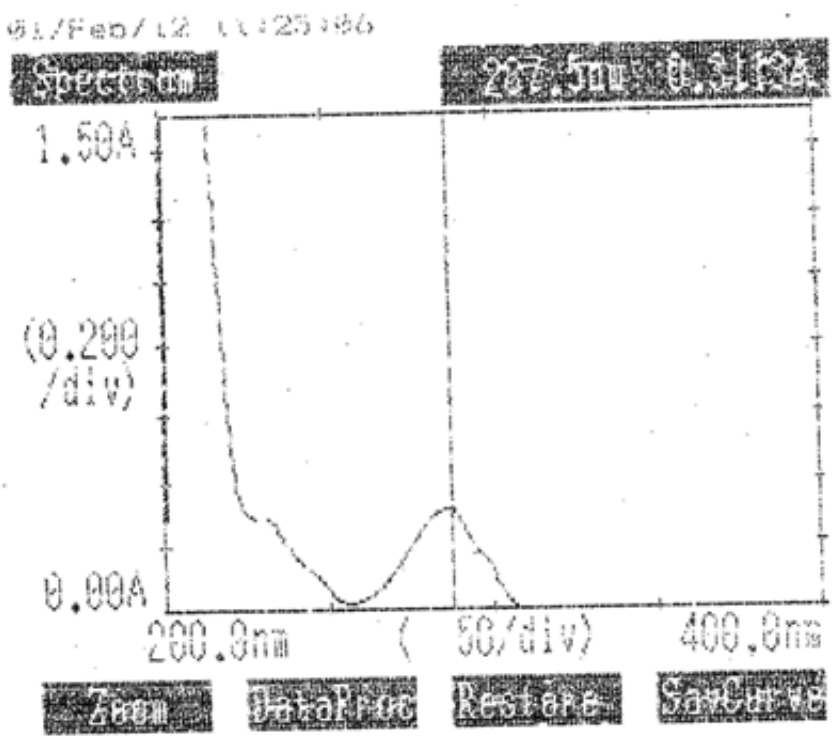


FIGURE 5

OVERLAIN SPECTRUM OF LAFUTIDINE AND DOMPERIDONE IN METHANOL

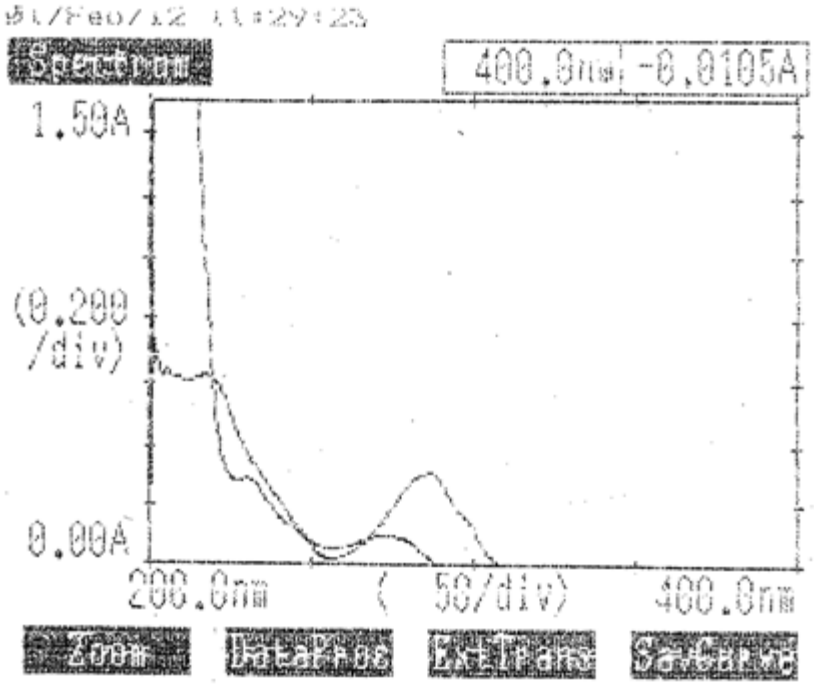


FIGURE 6

CALIBRATION CURVE OF LAFUTIDINE IN METHANOL AT 273 nm SIMULTANEOUS EQUATION METHOD

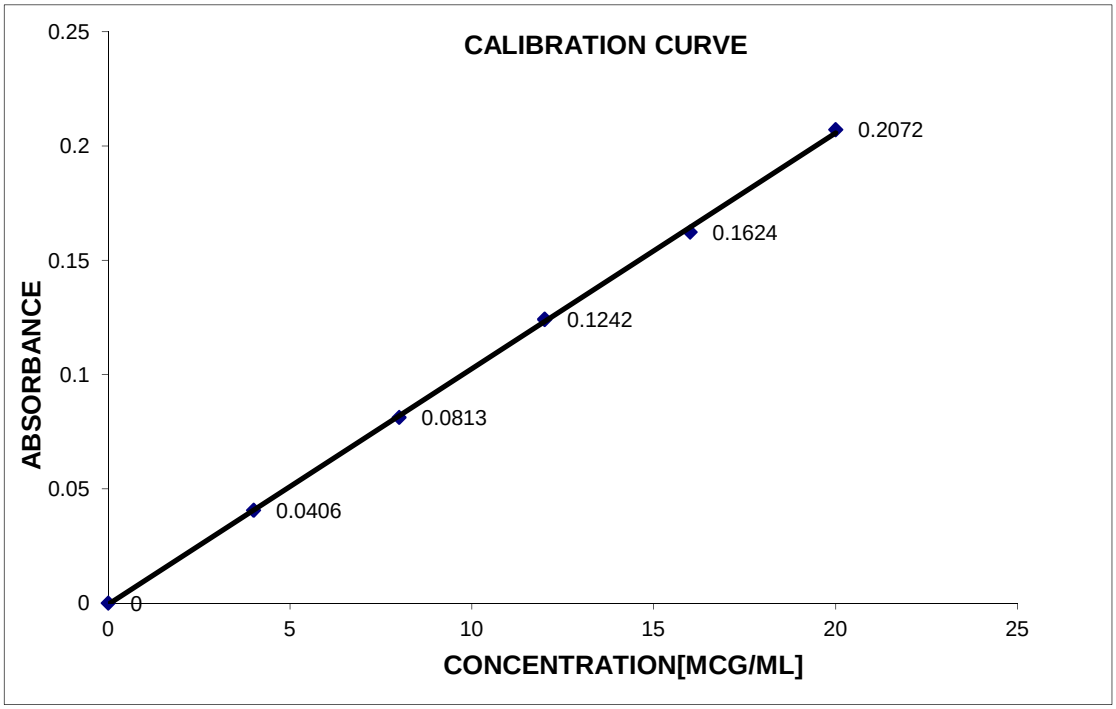


FIGURE 7

CALIBRATION CURVE OF LAFUTIDINE IN METHANOL AT 287.5 nm

SIMULTANEOUS EQUATION METHOD

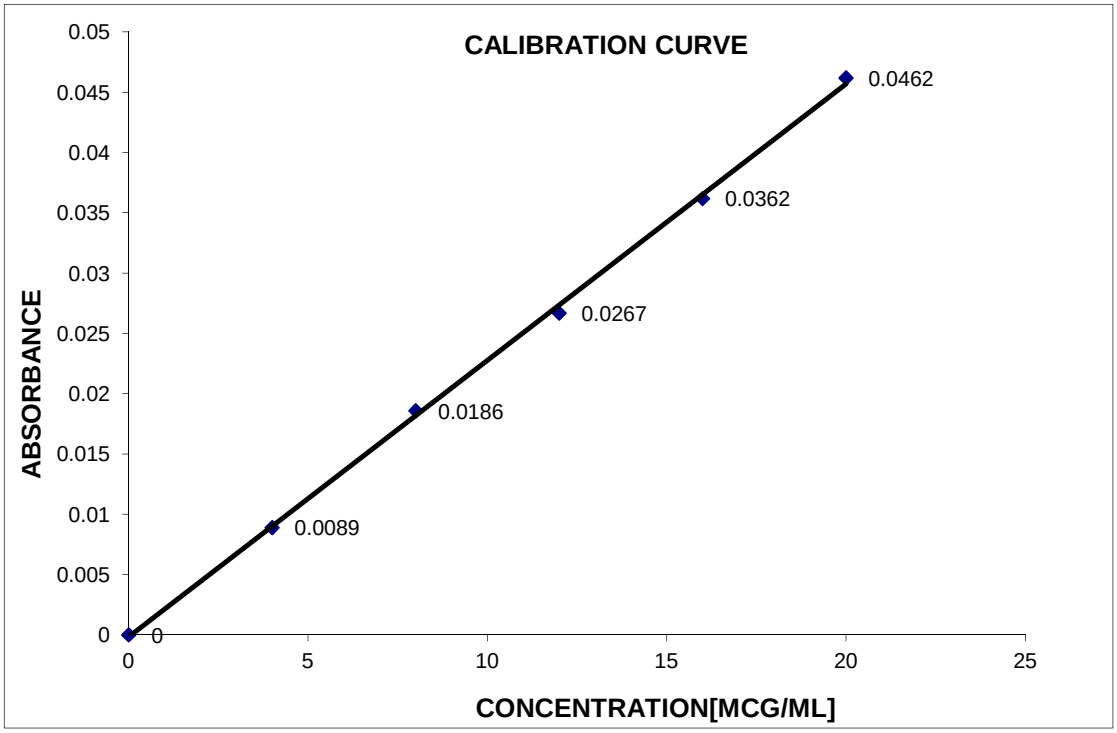


FIGURE 8

CALIBRATION CURVE OF DOMPERIDONE IN METHANOL AT 273 nm

SIMULTANEOUS EQUATION METHOD

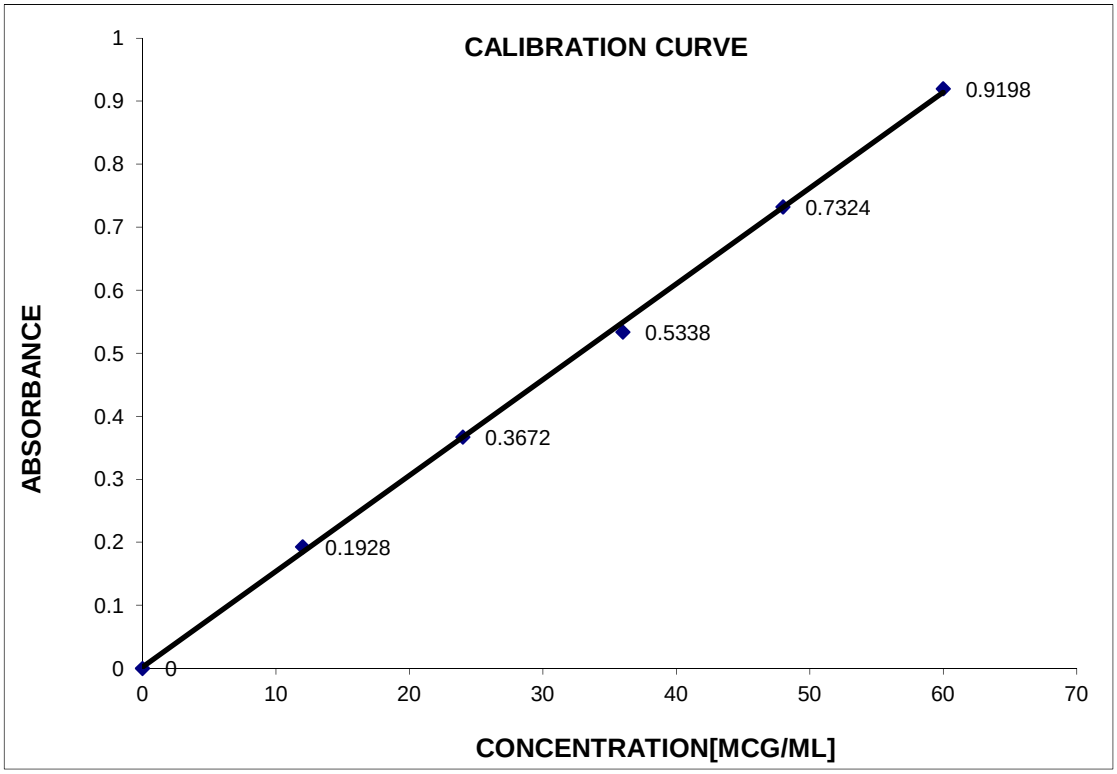


FIGURE 9

CALIBRATION CURVE OF DOMPERIDONE IN METHANOL AT 287.5 nm SIMULTANEOUS EQUATION METHOD

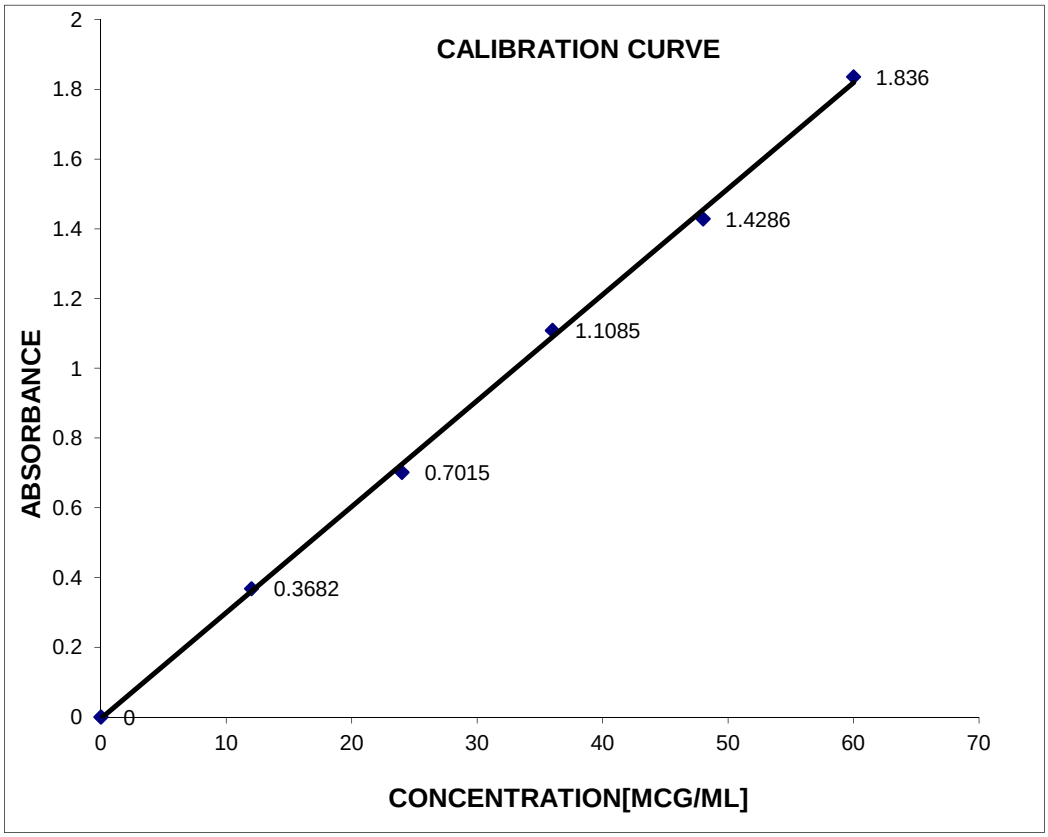


FIGURE 10

CALIBRATION CURVE OF LAFUTIDINE IN METHANOL AT 276-271 nm

AREA UNDER CURVE METHOD

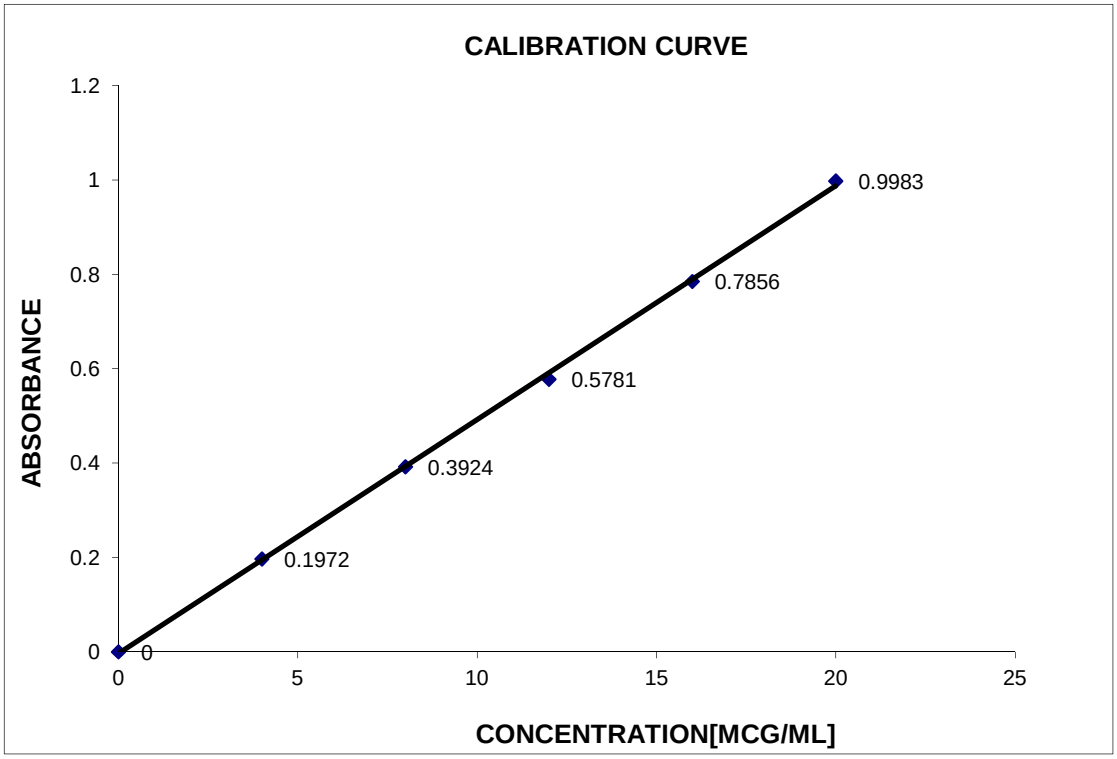


FIGURE 11

CALIBRATION CURVE OF LAFUTIDINE IN METHANOL AT 291-282 nm

AREA UNDER CURVE METHOD

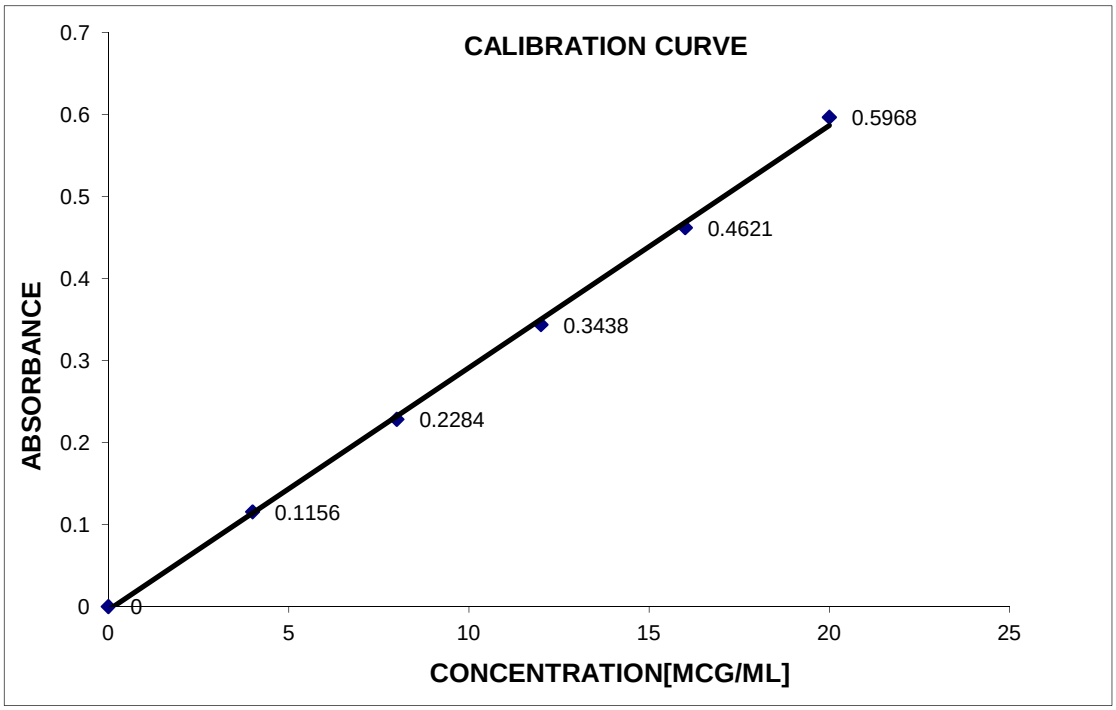


FIGURE 12

CALIBRATION CURVE OF DOMPERIDONE IN METHANOL AT 276-271 nm AREA UNDER CURVE METHOD

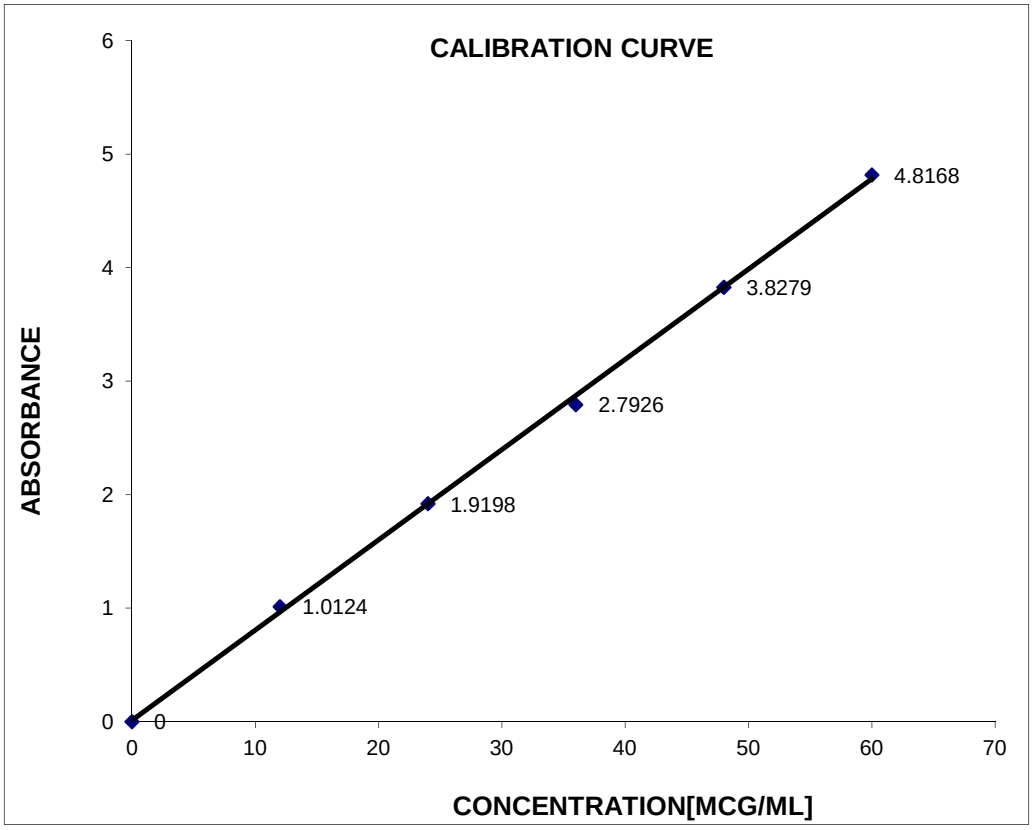


FIGURE 13

CALIBRATION CURVE OF DOMPERIDONE IN METHANOL AT 291-282 nm AREA UNDER CURVE METHOD

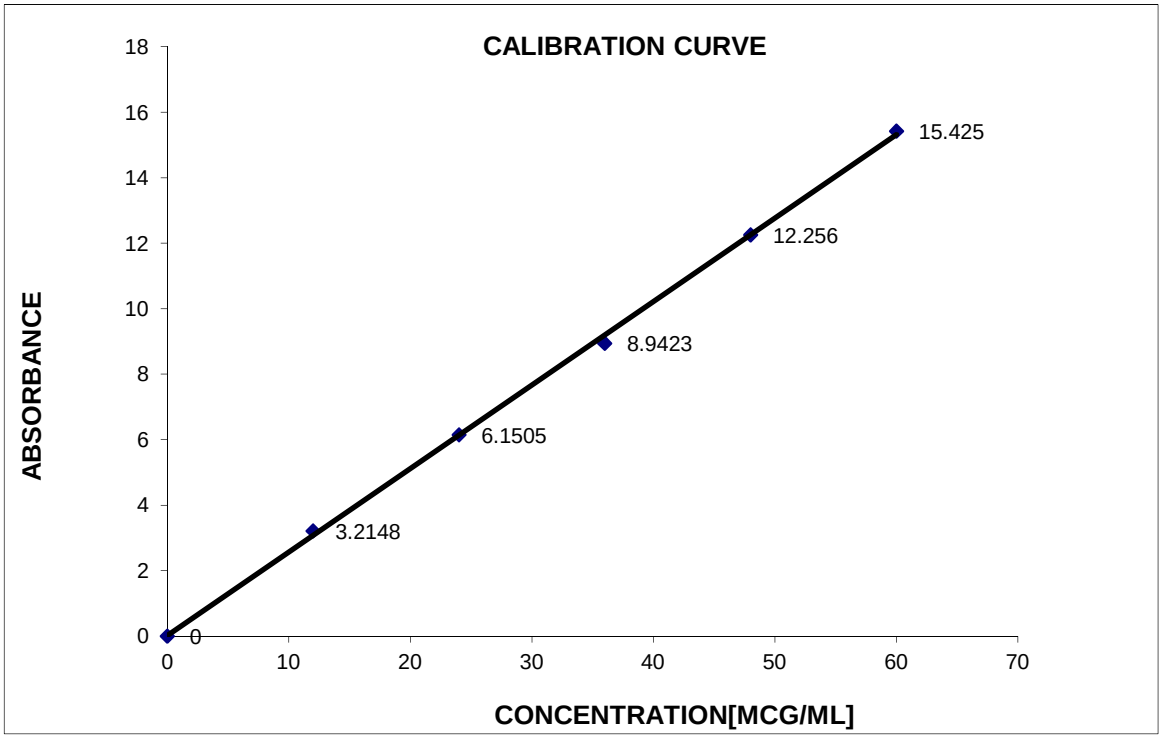
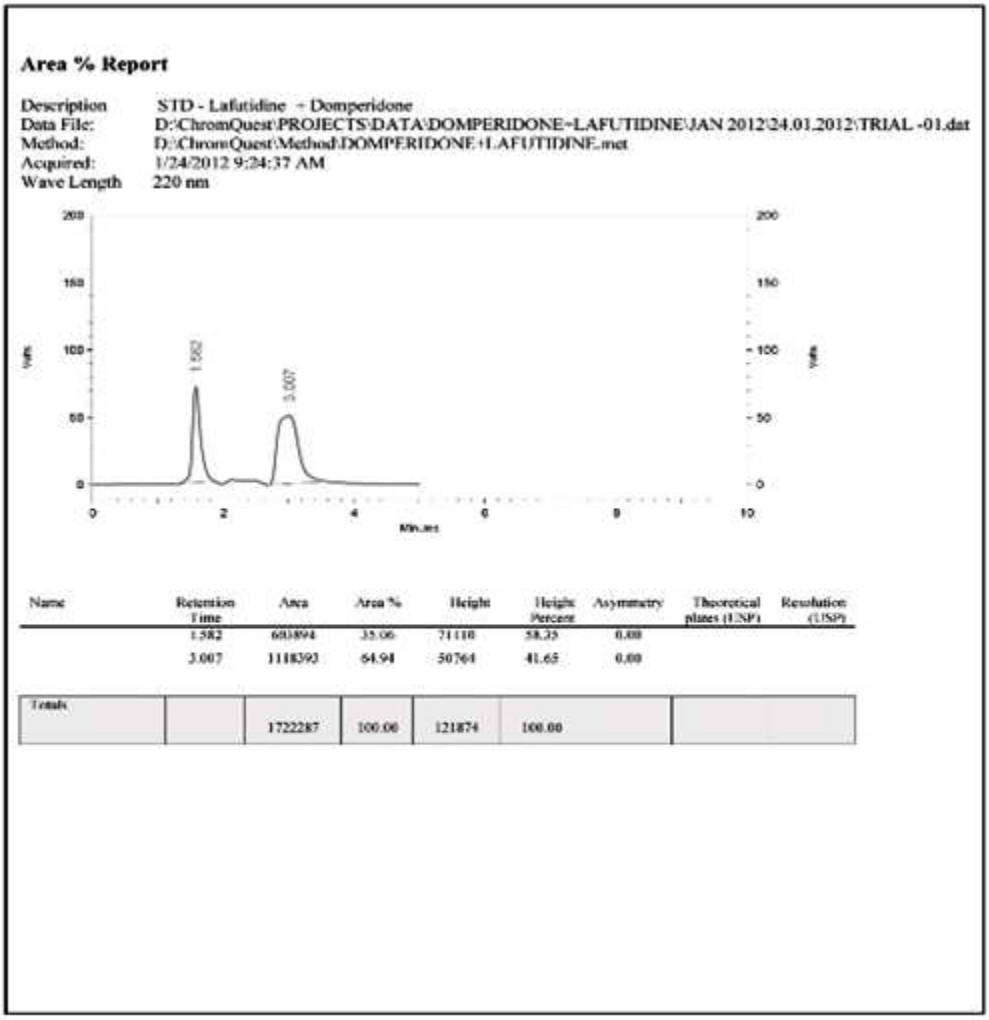


FIGURE 14

INITIAL SEPARATION CONDITIONS IN METHANOL: WATER

50:50%V/V



Area % Report

Description STD - Lafutidine + Domperidone
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\TRIAL -01.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/24/2012 9:24:37 AM
Wave Length 220 nm

Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
	1.582	603894	35.06	71110	58.35	0.00		
	3.007	1118393	64.94	50764	41.65	0.00		
Totals		1722287	100.00	121874	100.00			

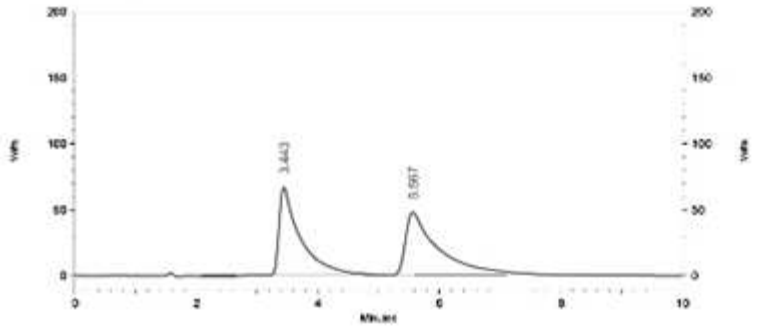
FIGURE 15

EFFECT OF RATIO OF MOBILE PHASE

METHANOL: 0.5% AMMONIUM ACETATE BUFFER (50:50 % V/V)

Area % Report

Description: STD - Lafutidine + Domperidone
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\TRIAL -02.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/24/2012 10:53:37 AM
Wave Length: 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	3.443	1634265	47.97	66643	58.31	3.61	597	0.00
DOMPERIDONE	5.567	1772840	52.03	47656	41.69	3.57	668	2.98
Totals		3407105	100.00	114299	100.00			

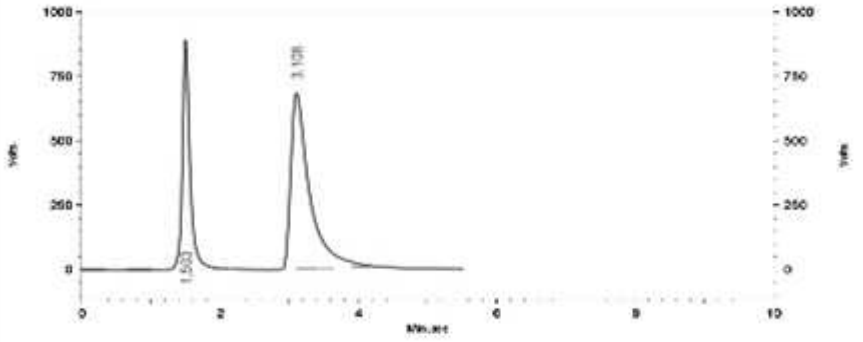
FIGURE 16

EFFECT OF RATIO OF MOBILE PHASE

METHANOL: 0.5% AMMONIUM ACETATE BUFFER (60:40 % V/V)

Area % Report

Description STD - Lafutidine + Domperidone
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\TRIAL -03.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/24/2012 11:53:17 AM
Wave Length 220 nm



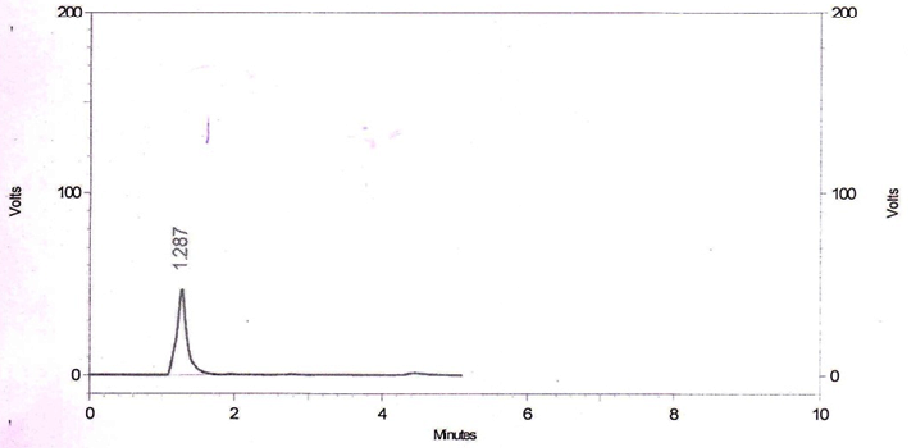
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
	1.503	6807599	34.54	888485	56.60	0.00	756	0.00
	3.108	12901231	65.46	681127	43.40	0.00	1456	4.52
Totals		19708830	100.00	1569532	100.00			

FIGURE 17

INITIAL SEPARATION OF LAFUTIDINE IN MOBILE PHASE

Area % Report

Description Lafutidine - std
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\A_001_LAFUTIDINE.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/24/2012 8:59:50 PM
Wave Length 220 nm



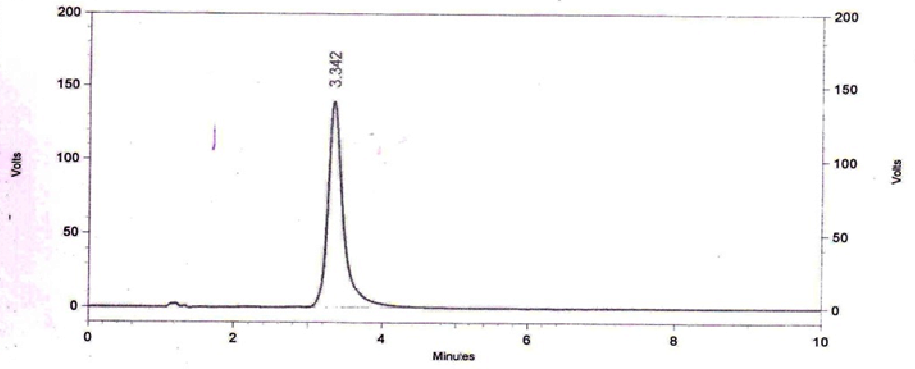
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.287	455793	100.00	46692	100.00	1.25	1084	0.00
Totals		455793	100.00	46692	100.00			

FIGURE 18

INITIAL SEPARATION OF DOMPERIDONE IN MOBILE PHASE

Area % Report

Description Domperidone - std
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\0001_DOMPERIDONE.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/25/2012 2:32:14 AM
Wave Length 220 nm



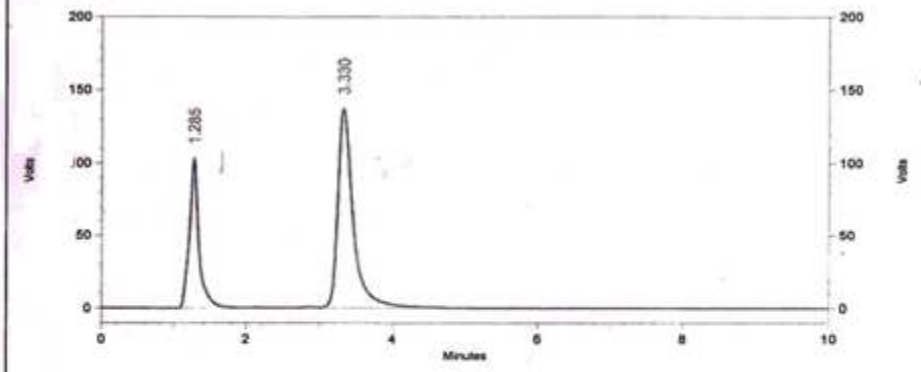
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
DOMPERIDONE	3.342	2016010	100.00	139026	100.00	1.53	1513	0.00
Totals		2016010	100.00	139026	100.00			

FIGURE 19

OPTIMIZED CHROMATOGRAM OF LAFUTIDINE AND DOMPERIDONE IN MOBILE PHASE

Area % Report

Description STD - Lafutidine (50mcg/mL) + Domperidone (150mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\002_SS-01.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/24/2012 9:29:17 PM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.285	938519	32.23	102930	42.97	1.28	1059	0.00
DOMPERIDONE	3.330	1973359	67.77	136596	57.03	1.72	1461	7.23
Totals		2911878	100.00	239526	100.00			

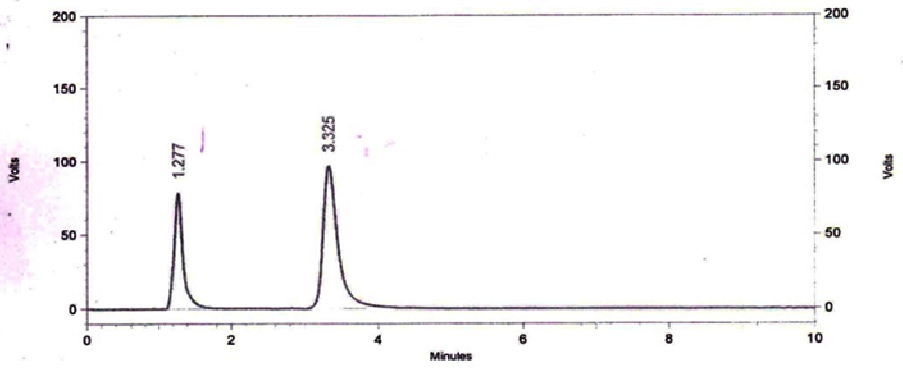
FIGURE 20

LINEARITY CHROMATOGRAM OF LAFUTIDINE AND DOMPERIDONE

(35, 105 µg/ mL)

Area % Report

Description STD 70% CONCENTRATION- Lafutidine (35mcg/mL) + Domperidone (105mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\007_LINERTY_70%.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/24/2012 10:27:28 PM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.277	665433	33.01	78134	44.82	1.41	1017	0.00
DOMPERIDONE	3.325	1350444	66.99	96177	55.18	1.66	1521	7.49
Totals		2015877	100.00	174311	100.00			

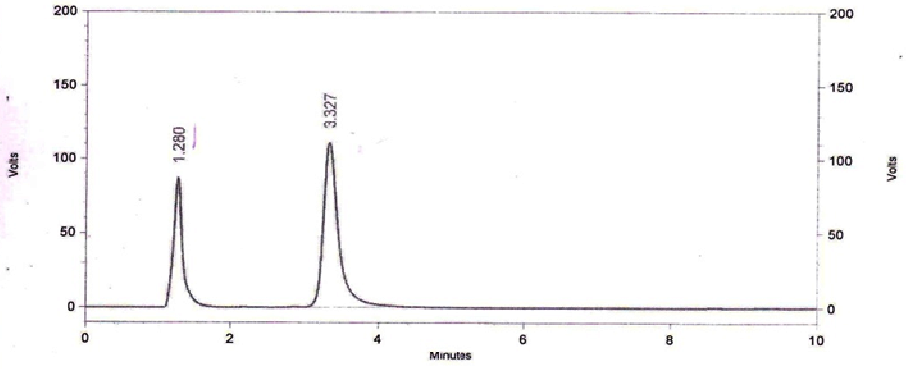
FIGURE 21

LINEARITY CHROMATOGRAM OF LAFUTIDINE AND DOMPERIDONE

(40, 120 µg/ mL)

Area % Report

Description STD 80% CONCENTRATION- Lafutidine (40mcg/mL) + Domperidone (120mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\008_LINERTY_80%.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/24/2012 10:39:07 PM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.280	759021	32.67	86628	43.99	1.36	1084	0.00
DOMPERIDONE	3.327	1564025	67.33	110300	56.01	1.65	1515	7.39
Totals		2323046	100.00	196928	100.00			

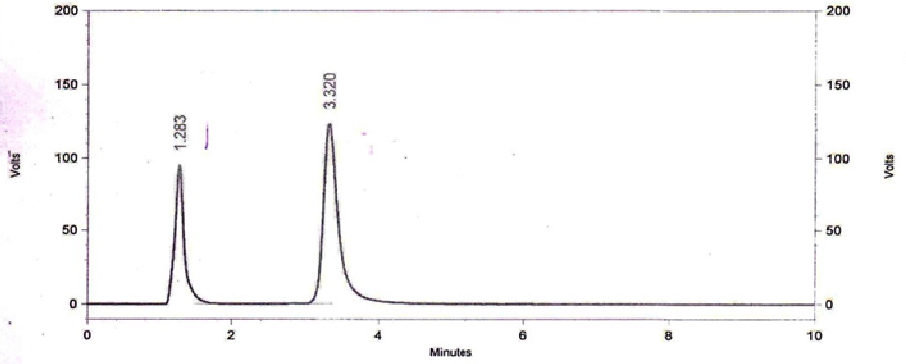
FIGURE 22

LINEARITY CHROMATOGRAM OF LAFUTIDINE AND DOMPERIDONE

(45, 135 µg/ mL)

Area % Report

Description STD 90% CONCENTRATION- Lafutidine (45mcg/mL) + Domperidone (135mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\009_LINERTY_90%.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/24/2012 10:50:47 PM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.283	849833	32.57	94695	43.50	1.32	1061	0.00
DOMPERIDONE	3.320	1759688	67.43	122999	56.50	1.63	1496	7.27
Totals		2609521	100.00	217694	100.00			

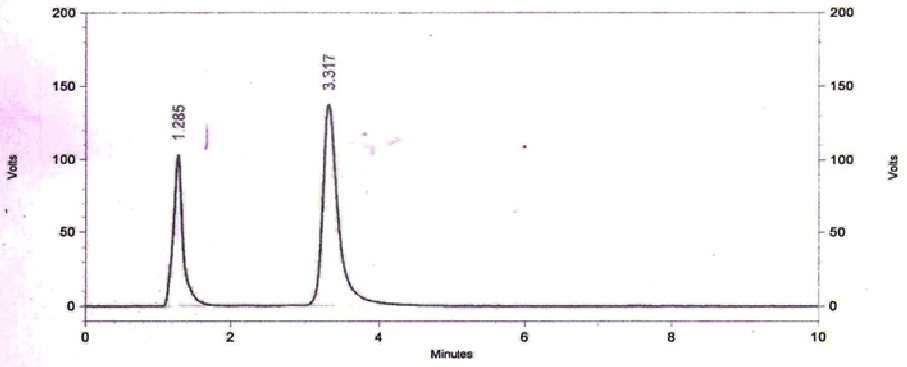
FIGURE 23

LINEARITY CHROMATOGRAM OF LAFUTIDINE AND DOMPERIDONE

(50, 150 µg/ mL)

Area % Report

Description STD 100% CONCENTRATION- Lafutidine (50mcg/mL) + Domperidone (150mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\010_LINERTY_100%.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/24/2012 11:02:26 PM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.285	946271	32.48	102748	42.88	1.29	1038	0.00
DOMPERIDONE	3.317	1967133	67.52	136890	57.12	1.64	1489	7.19
Totals		2913404	100.00	239638	100.00			

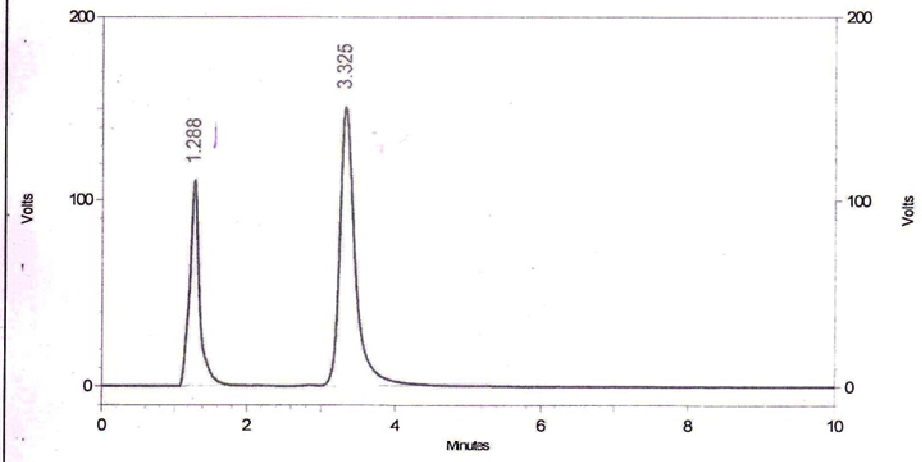
FIGURE 24

LINEARITY CHROMATOGRAM OF LAFUTIDINE AND DOMPERIDONE

(55, 165 µg/ mL)

Area % Report

Description STD 110% CONCENTRATION- Lafutidine (55mcg/mL) + Domperidone (165mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\011_LINERTY_110%.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/24/2012 11:14:04 PM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.288	1037244	32.37	110250	42.27	1.25	1023	0.00
DOMPERIDONE	3.325	2166712	67.63	150567	57.73	1.63	1488	7.14
Totals		3203956	100.00	260817	100.00			

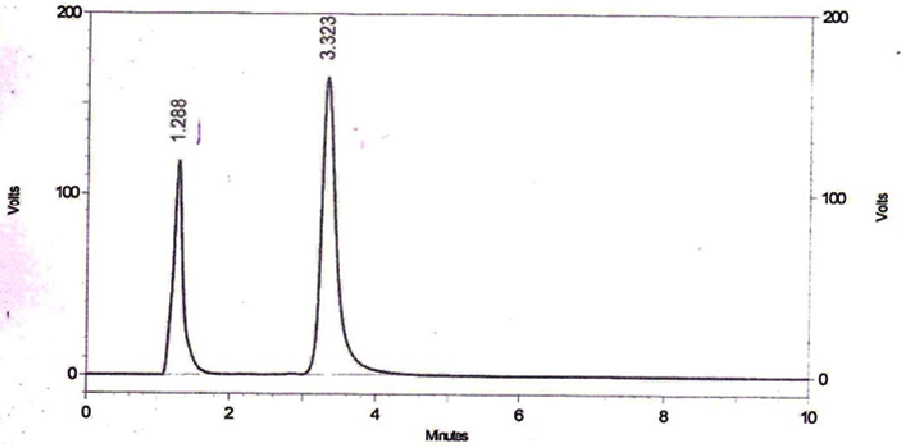
FIGURE 25

LINEARITY CHROMATOGRAM OF LAFUTIDINE AND DOMPERIDONE

(60, 180 µg/ mL)

Area % Report

Description STD 120% CONCENTRATION- Lafutidine (60mcg/mL) + Domperidone (180mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\012_LINERTY_120%.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/24/2012 11:25:44 PM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.288	1134119	32.30	117647	41.80	1.24	1001	0.00
DOMPERIDONE	3.323	2376688	67.70	163838	58.20	1.63	1472	7.06
Totals		3510807	100.00	281485	100.00			

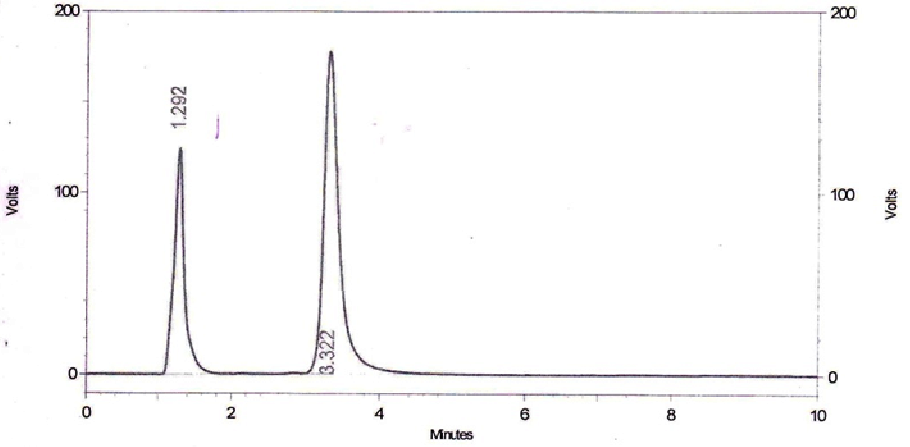
FIGURE 26

LINEARITY CHROMATOGRAM OF LAFUTIDINE AND DOMPERIDONE

(65, 195 µg/ mL)

Area % Report

Description STD 130% CONCENTRATION- Lafutidine (65mcg/mL) + Domperidone (195mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\013_LINERTY_130%.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/24/2012 11:37:23 PM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.292	1227637	32.27	123991	41.21	1.21	1088	0.00
DOMPERIDONE	3.322	2576467	67.73	176916	58.79	1.61	1452	6.97
Totals		3804104	100.00	300907	100.00			

FIGURE 27

CALIBRATION CURVE OF LAFUTIDINE RP-HPLC

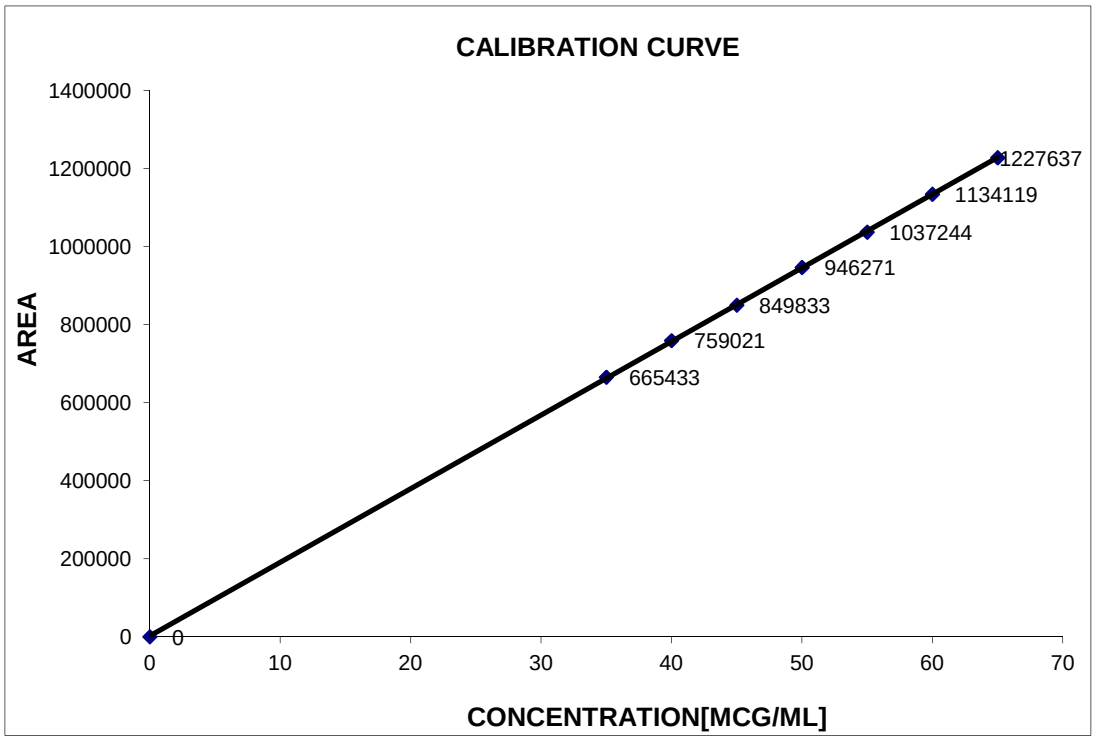


FIGURE 28

CALIBRATION CURVE OF DOMPERIDONE RP-HPLC

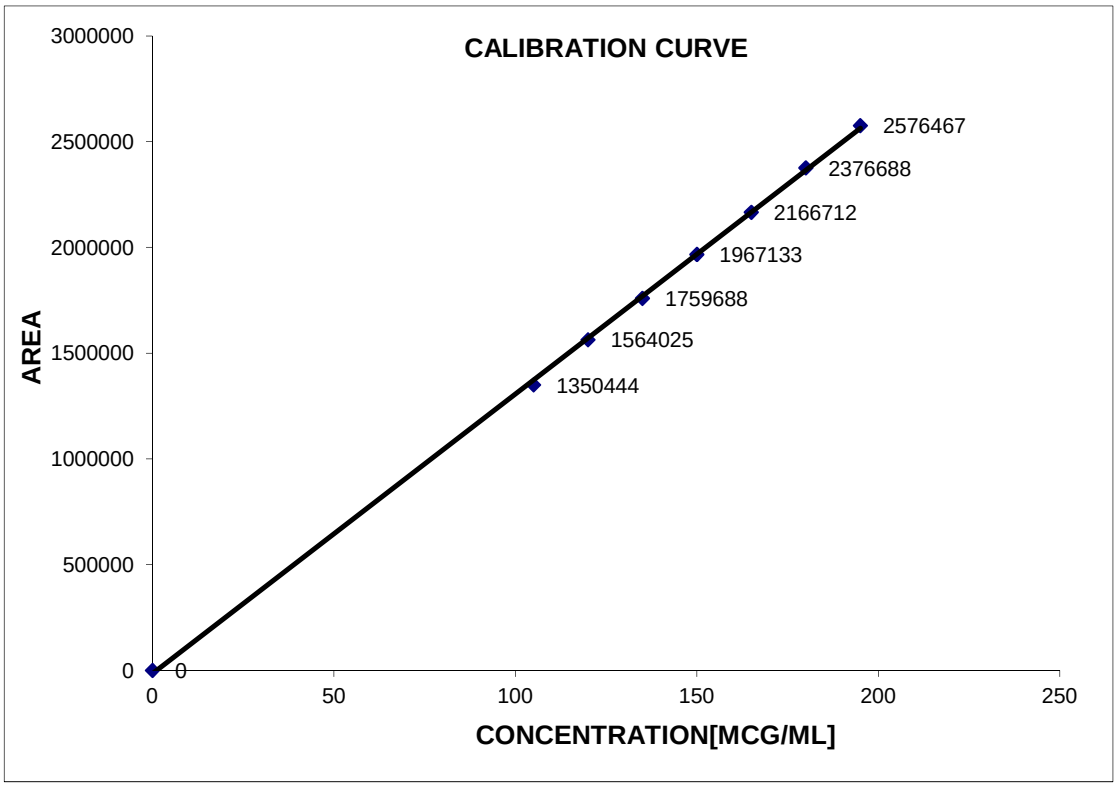


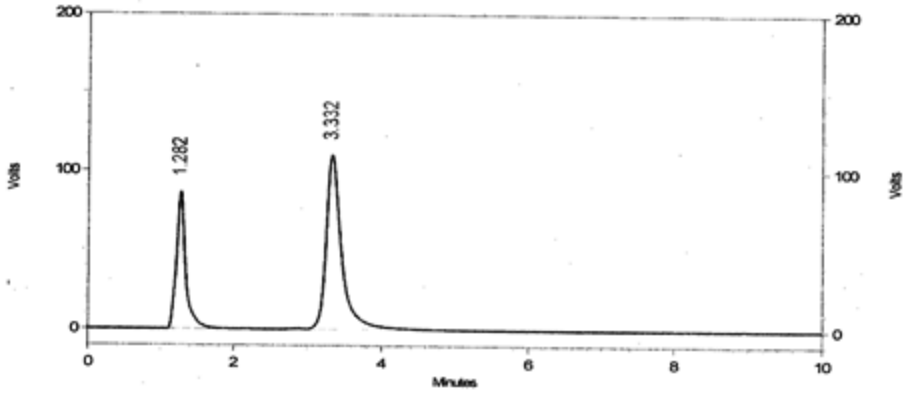
FIGURE 29

CHROMATOGRAM FOR ANALYSIS OF FORMULATION LAFAXID-D

REPEATABILITY – 1

Area % Report

Description SPL- 01_Lafaxid-D - LOW LEVEL CONCENTRATION - 80%
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\014_P&A_L1.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/24/2012 11:48:58 PM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.282	757585	32.62	86114	43.99	1.36	1076	0.00
DOMPERIDONE	3.332	1564651	67.38	109626	56.01	1.62	1525	7.39
Totals		2322236	100.00	195740	100.00			

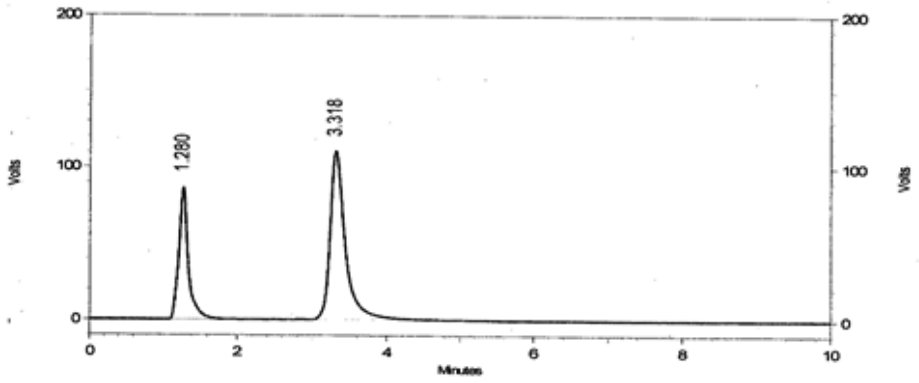
FIGURE 30

CHROMATOGRAM FOR ANALYSIS OF FORMULATION LAFAXID- D

REPEATABILITY – 2

Area % Report

Description SPL- 02_Lafaxid-D - LOW LEVEL CONCENTRATION - 80%
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\015_P&A_L2.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/25/2012 12:00:37 AM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.280	760232	32.70	86150	43.93	1.37	1074	0.00
DOMPERIDONE	3.318	1564736	67.30	109969	56.07	1.60	1511	7.34
Totals		2324968	100.00	196119	100.00			

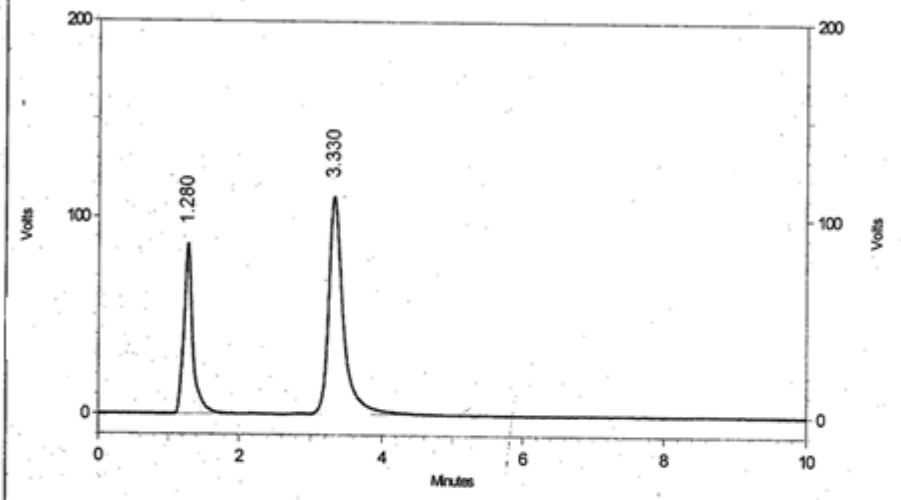
FIGURE 31

CHROMATOGRAM FOR ANALYSIS OF FORMULATION LAFAXID- D

REPEATABILITY – 3

Area % Report

Description SPL- 03_Lafaxid-D - LOW LEVEL CONCENTRATION - 80%
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\016_P&A_L3.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/25/2012 12:12:16 AM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.280	756676	32.59	85917	43.86	1.37	1070	0.00
DOMPERIDONE	3.330	1565057	67.41	109983	56.14	1.59	1529	7.39
Totals		2321733	100.00	195900	100.00			

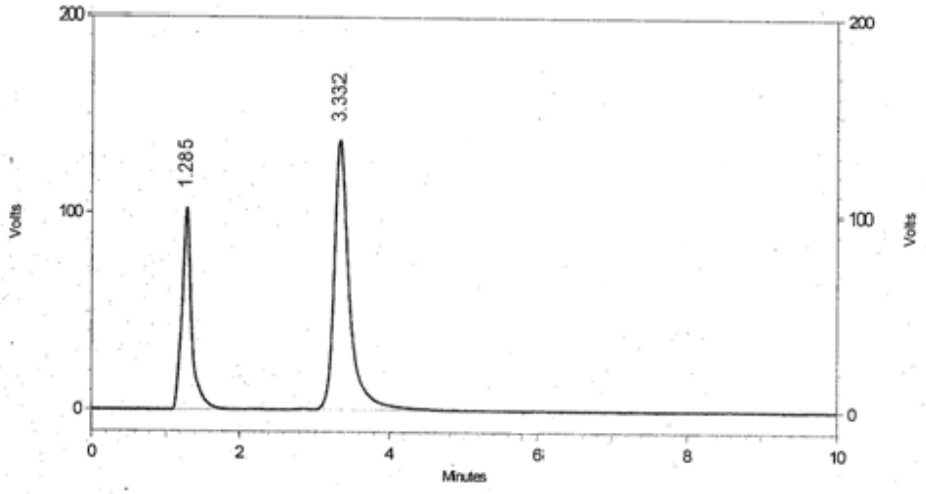
FIGURE 32

CHROMATOGRAM FOR ANALYSIS OF FORMULATION LAFAXID- D

REPEATABILITY – 4

Area % Report

Description SPL- 01_Lafaxid-D - MIDDLE LEVEL CONCENTRATION - 100%
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\017_P&A_M1.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/25/2012 12:23:56 AM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.285	945770	32.39	102072	42.84	1.29	1029	0.00
DOMPERIDONE	3.332	1974102	67.61	136201	57.16	1.58	1492	7.20
Totals		2919872	100.00	238273	100.00			

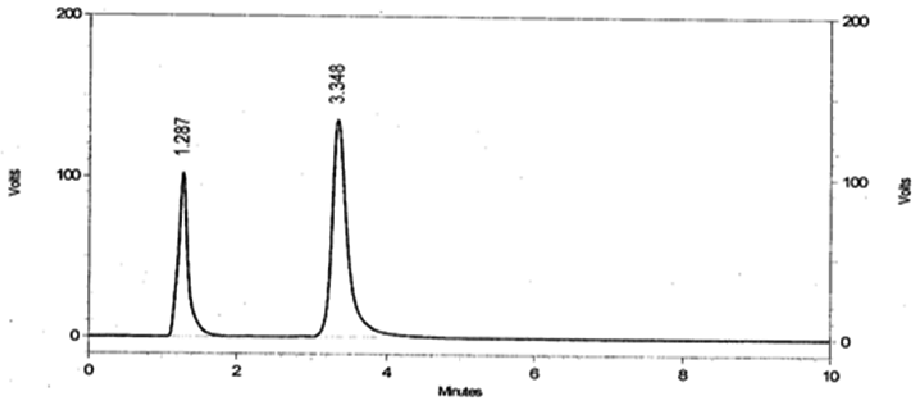
FIGURE 33

CHROMATOGRAM FOR ANALYSIS OF FORMULATION LAFAXID- D

REPEATABILITY – 5

Area % Report

Description SPL- 02_Lafaxid-D - MIDDLE LEVEL CONCENTRATION - 100%
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\018_P&A_M2.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/25/2012 12:35:35 AM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.287	944302	32.67	101846	42.99	1.29	1028	0.00
DOMPERIDONE	3.348	1946002	67.33	135081	57.01	1.56	1493	7.23
Totals		2890304	100.00	236927	100.00			

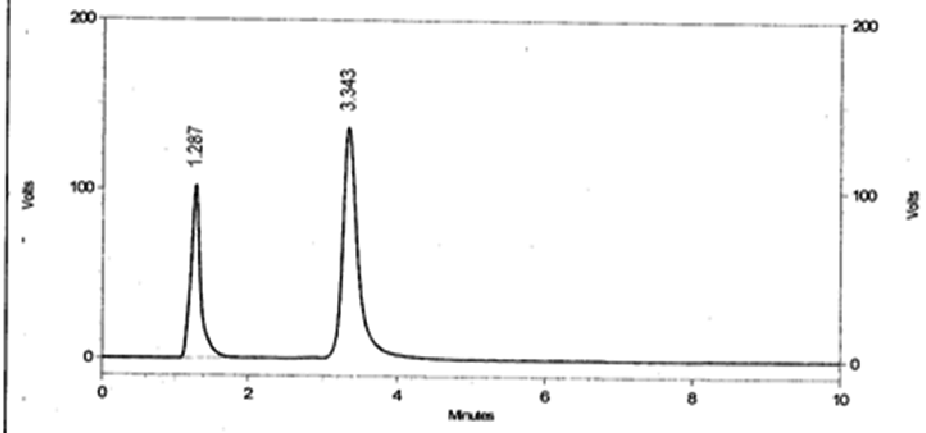
FIGURE 34

CHROMATOGRAM FOR ANALYSIS OF FORMULATION LAFAXID- D

REPEATABILITY – 6

Area % Report

Description SPL- 03_Lafaxid-D - MIDDLE LEVEL CONCENTRATION - 100%
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\019_P&A_M3.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/25/2012 12:47:13 AM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.287	946407	32.48	101904	42.86	1.29	1025	0.00
DOMPERIDONE	3.343	1967715	67.52	135860	57.14	1.57	1497	7.21
Totals		2914122	100.00	237764	100.00			

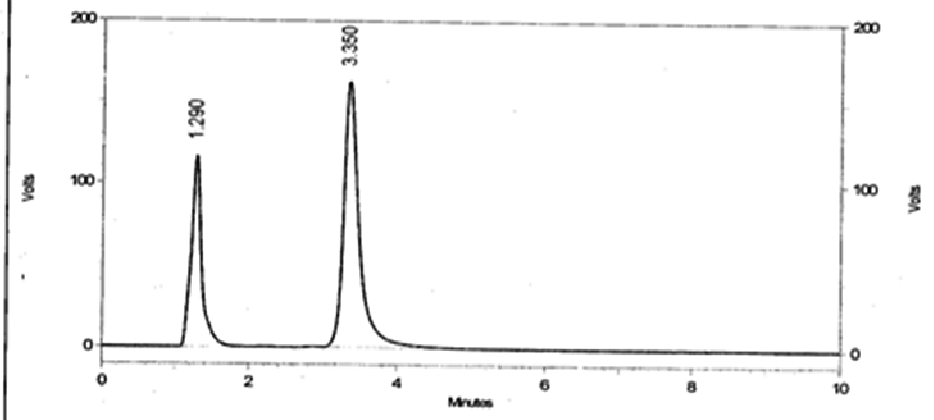
FIGURE 35

CHROMATOGRAM FOR ANALYSIS OF FORMULATION LAFAXID- D

REPEATABILITY – 7

Area % Report

Description SPL- 01_Lafaxid-D - HIGH LEVEL CONCENTRATION - 120%
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\020_P&A_H1.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/25/2012 12:58:56 AM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.290	1127469	32.15	115800	41.71	1.24	1081	0.00
DOMPERIDONE	3.350	2379363	67.85	161801	58.29	1.56	1476	7.06
Totals		3506832	100.00	277601	100.00			

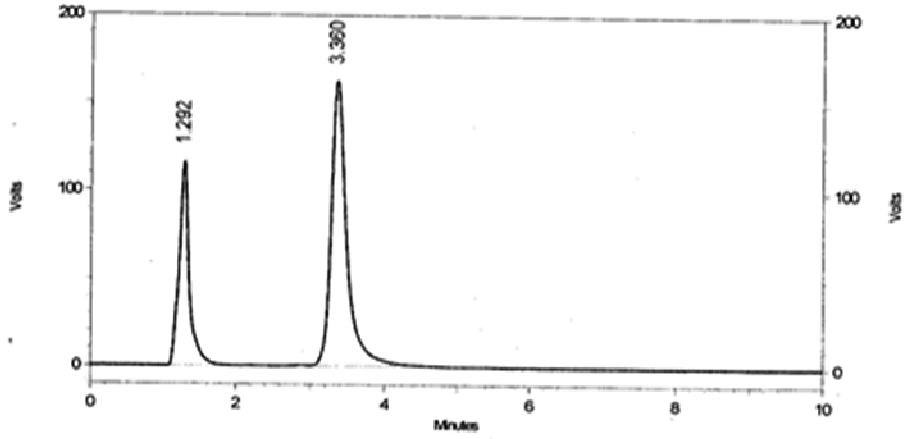
FIGURE 36

CHROMATOGRAM FOR ANALYSIS OF FORMULATION LAFAXID- D

REPEATABILITY – 8

Area % Report

Description SPL- 02_Lafaxid-D - HIGH LEVEL CONCENTRATION - 120%
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\021_P&A_H2.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/25/2012 1:10:34 AM
Wave Length 220 nm

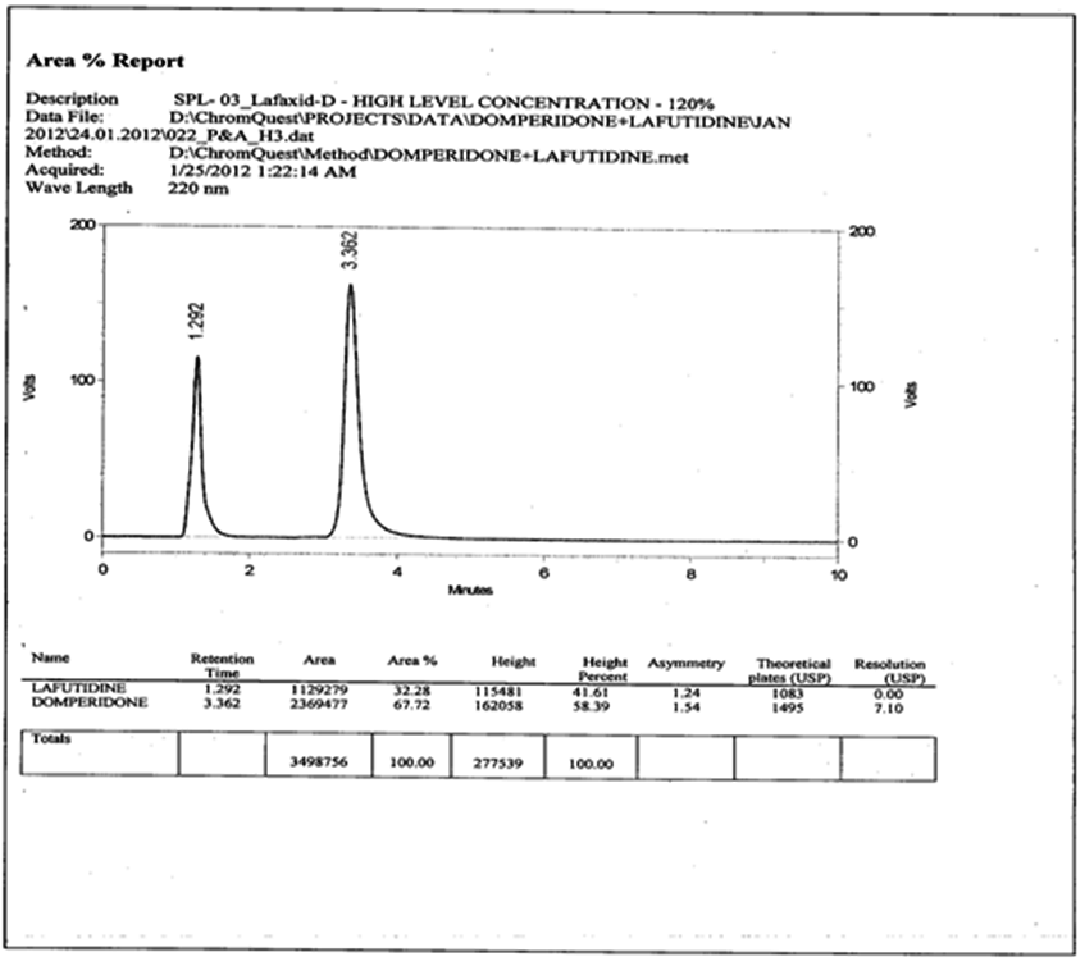


Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.292	1127399	32.36	115488	41.66	1.24	1087	0.00
DOMPERIDONE	3.360	2356545	67.64	161756	58.34	1.55	1495	7.11
Totals		3483944	100.00	277244	100.00			

FIGURE 37

CHROMATOGRAM FOR ANALYSIS OF FORMULATION LAFAXID- D

REPEATABILITY – 9



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.292	1129279	32.28	115481	41.61	1.24	1083	0.00
DOMPERIDONE	3.362	2369477	67.72	162058	58.39	1.54	1495	7.10
Totals		3498756	100.00	277539	100.00			

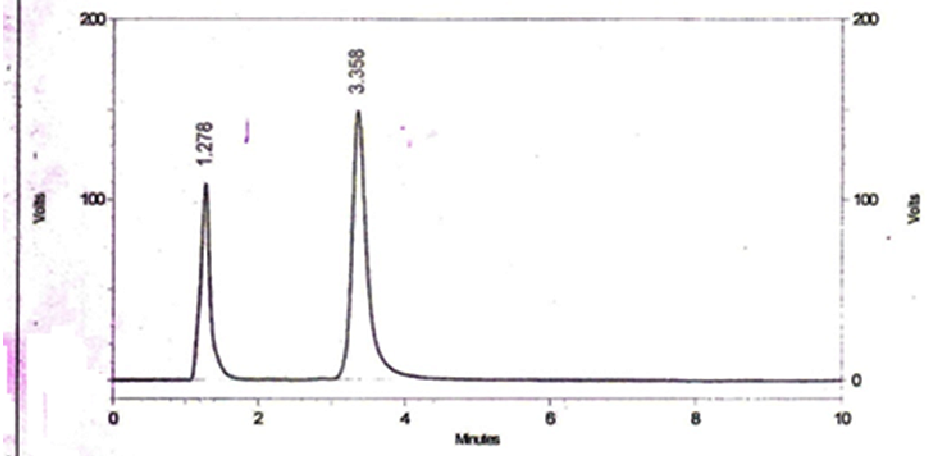
FIGURE 38

CHROMATOGRAM FOR FIRST RECOVERY OF FORMULATION

LAFAXID- D

Area % Report

Description SPL-RECOVERY- 01_Lafaxid-D - STANDARD 10%
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\023RECOVERY_10%.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/25/2012 1:33:53 AM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.278	1037201	32.46	108141	42.18	1.27	1095	0.00
DOMPERIDONE	3.358	2158114	67.54	148233	57.82	1.53	1505	7.22
Totals		3195315	100.00	256374	100.00			

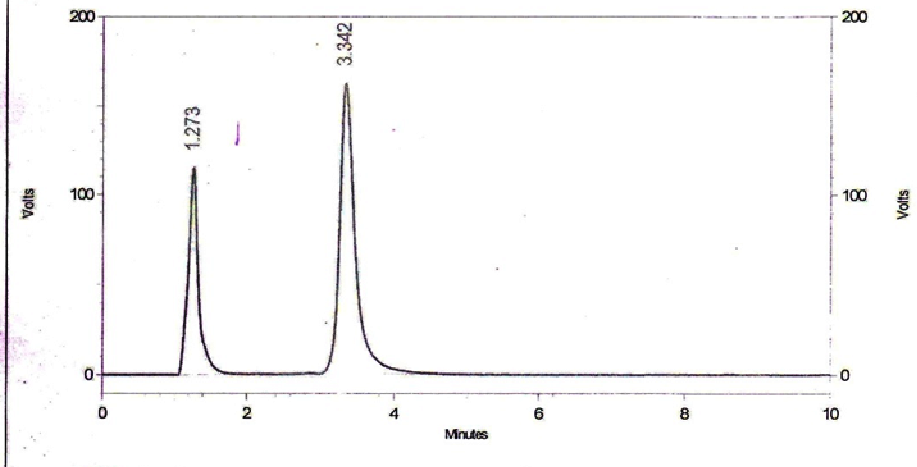
FIGURE 39

CHROMATOGRAM FOR SECOND RECOVERY OF FORMULATION

LAFAXID- D

Area % Report

Description SPL-RECOVERY- 02_Lafaxid-D - STANDARD 20%
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\025RECOVERY_20%.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/25/2012 1:45:36 AM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.273	1144623	32.47	114825	41.53	1.25	1061	0.00
DOMPERIDONE	3.342	2381089	67.53	161688	58.47	1.53	1471	7.06
Totals		3525712	100.00	276513	100.00			

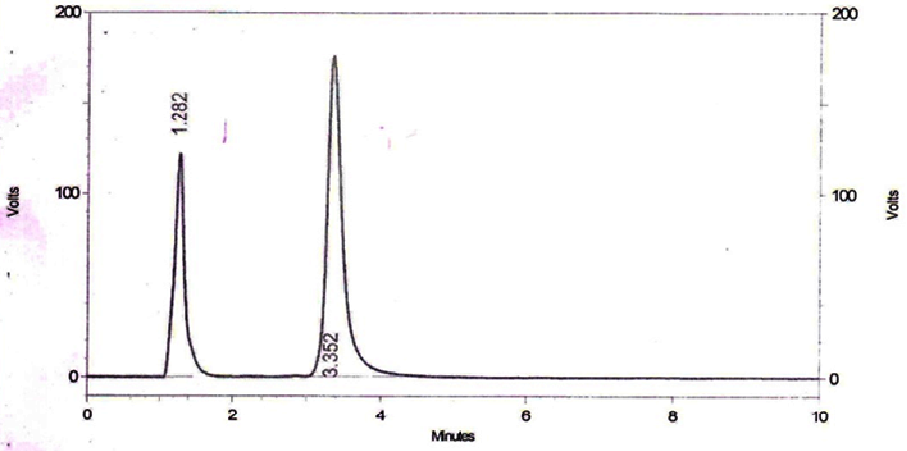
FIGURE 40

CHROMATOGRAM FOR THIRD RECOVERY OF FORMULATION

LAFAXID- D

Area % Report

Description SPL-RECOVERY- 03_Lafaxid-D - STANDARD 30%
Data File: D:\ChromQuest\PROJECTS\DATA\DOMPERIDONE+LAFUTIDINE\JAN 2012\24.01.2012\025RECOVERY_30%.dat
Method: D:\ChromQuest\Method\DOMPERIDONE+LAFUTIDINE.met
Acquired: 1/25/2012 1:57:18 AM
Wave Length 220 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
LAFUTIDINE	1.282	1240305	32.42	121391	40.93	1.22	1059	0.00
DOMPERIDONE	3.352	2585600	67.58	175203	59.07	1.54	1475	7.04
Totals		3825905	100.00	296594	100.00			

Tables

TABLE 1

SOLUBILITY PROFILE OF LAFUTIDINE AND DOMPERIDONE IN POLAR AND NON-POLAR SOLVENTS

S.No.	SOLVENTS	LAFUTIDINE 10 mg in mL of solvent	DOMPERIDONE 10 mg in mL of solvent
1	Acetone	Freely soluble (60 µL)	Soluble(200 µL)
2	Acetonitrile	Sparingly soluble(400 µL)	Insoluble(100 mL)
3	Benzene	Sparingly soluble(500 µL)	Insoluble(100 mL)
4	Butanol	Freely soluble(40 µL)	Slightly soluble(5 mL)
5	Carbon tetra chloride	Slightly soluble(1 mL)	Insoluble(100 mL)
6	Chloroform	Very soluble(10 µL)	Insoluble(100 mL)
7	Dichloromethane	Freely soluble(20 µL)	Insoluble(100 mL)
8	Distilled water	Insoluble(100 mL)	Insoluble(100 mL)
9	DMF	Freely soluble(30 µL)	Soluble(200 µL)
10	Ethyl acetate	Freely soluble(100 µL)	Insoluble(100 mL)
11	Hydrochloric acid (0.1M)	Sparingly soluble(400 µL)	Insoluble(100 mL)
12	Methanol	Freely soluble(30 µL)	Slightly Soluble(5 mL)
13	Sodium Hydroxide (0.1M)	Insoluble(100 mL)	Slightly soluble(6 mL)

TABLE 2

OPTICAL CHARACTERISTICS OF LAFUTIDINE

SIMULTANEOUS EQUATION METHOD

Parameters	**At 273 nm**	**At 287.5 nm**
Beers law limit (μg mL^{-1})	4 – 20	4 – 20
Sandell's sensitivity (μg/cm^2/0.001 A.U)	0.096932	0.436137
Molar absorptivity	4428.4222	982.4955
Correlation coefficient (r)	0.99978	0.99966
Régression equation (y = mx+c)	y = 0.010316x - 0.00055	y = 0.002293x - 0.00016
Slope (m)	0.010316	0.002293
Intercept (c)	- 0.00055	-0.00016
LOD (μg mL^{-1})	0.1623455	1.538328
LOQ (μg mL^{-1})	0.491956	4.661601
Standard Error	0.001429	0.0005

TABLE 3

OPTICAL CHARACTERISTICS OF DOMPERIDONE

SIMULTANEOUS EQUATION METHOD

PARAMETERS	**AT 273 nm**	**AT 287.5 nm**
Beers law limit (μg mL^{-1})	12 - 60	12 – 60
Sandell's sensitivity (μg/cm^2/0.001 A.U)	0.065785	0.032894
Molar absorptivity	6544.005	12739.982
Correlation coefficient (r)	0.9997	0.99958
Régression equation (y = m*x*+c)	y = 0.015201x +0.001638	y = 0.0304x - 0.00488
Slope (m)	0.015201	0.0304
Intercept (c)	0.001638	-0.00488
LOD (μg mL^{-1})	0.0202818	0.268180
LOQ (μg mL^{-1})	0.0614602	0.8126696
Standard Error	0.009294	0.022013

TABLE 4

QUANTIFICATION OF FORMULATION (LAFAXID-D)

SIMULTANEOUS EQUATION METHOD

Drug	Sample No.	Labeled amount* (mg/tab)	Amount found* (mg/tab)	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
LAF	1	10	9.849	98.49	98.536	1.14185	1.1588	0.04567
	2	10	9.948	99.48				
	3	10	9.738	97.38				
	4	10	9.745	97.45				
	5	10	9.988	99.88				
DOM	1	30	29.98	99.93	99.88	0.4658	0.4664	0.01863
	2	30	29.86	99.53				
	3	30	29.79	99.3				
	4	30	30.108	100.36				
	5	30	30.09	100.3				

* Mean of five observations

TABLE 5

INTRADAY AND INTERDAY ANALYSIS OF FORMULATION (LAFAXID-D) SIMULTANEOUS EQUATION METHOD

Drug	Sample No.	Labeled amount (mg/ tab)	Percentage obtained*		S.D.		% R.S.D.		Standard error	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
LAF	1	10	98.04	98.84	0.0500	0.8150	0.0510	0.8245	0.0056	0.0906
	2	10	98.09	99.67						
	3	10	98.14	98.04						
Mean			98.09	98.815						
DOM	1	30	99.18	99.87	0.6149	0.6413	0.6245	0.6468	0.0683	0.0713
	2	30	98.18	98.94						
	3	30	98.06	98.64						
Mean			98.47	99.15						

* Mean of five observations

TABLE 6

RUGGEDNESS STUDY (LAFAXID-D)

SIMULTANEOUS EQUATION METHOD

Drug	Condition	Sample No.	Amount found (mg/ tab)*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
LAF	Analyst-1	1	9.899	98.99	98.974	0.9323	0.9419	0.03729
		2	9.969	99.69				
		3	9.754	97.54				
		4	9.99	99.9				
		5	9.875	98.75				
DOM	Analyst-1	1	29.98	99.93	99.944	0.2871	0.2872	0.01148
		2	29.88	99.6				
		3	29.92	99.73				
		4	30.06	100.2				
		5	30.08	100.26				

* Mean of five observations

TABLE 7

RUGGEDNESS STUDY (LAFAXID-D)
SIMULTANEOUS EQUATION METHOD

Drug	Condition	Sample No.	Amount found (mg/ tab)*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
LAF	Analyst-2	1	9.919	99.19	99.562	0.3119	0.3133	0.0124
		2	9.982	99.82				
		3	9.99	99.9				
		4	9.96	99.6				
		5	9.93	99.3				
DOM	Analyst-2	1	30.06	100.2	99.812	0.2833	0.2838	0.01133
		2	30.01	100.03				
		3	29.88	99.6				
		4	29.89	99.63				
		5	29.88	99.6				

* Mean of five observations

TABLE 8

RUGGEDNESS STUDY (LAFAXID-D)

SIMULTANEOUS EQUATION METHOD

Drug	Condition	Sample No.	Amount found (mg/ tab)*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
LAF	Instrument-1	1	9.8694	98.69	99.242	0.9494	0.9566	0.0379
		2	10.05	100.5				
		3	9.832	98.32				
		4	9.87	98.70				
		5	10.00	100.00				
DOM	Instrument-1	1	30.03	100.1	99.88	0.4438	0.4443	0.0177
		2	30.05	100.16				
		3	29.86	99.53				
		4	30.1	100.33				
		5	29.79	99.3				

* Mean of five observations

TABLE 9

RUGGEDNESS STUDY (LAFAXID-D)

SIMULTANEOUS EQUATION METHOD

Drug	Condition	Sample No.	Amount found (mg/ tab)*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
LAF	Instrument-2	1	9.929	99.29	98.848	0.8112	0.8206	0.0324
		2	9.949	99.49				
		3	9.934	99.34				
		4	9.754	97.54				
		5	9.858	98.58				
DOM	Instrument-2	1	30.02	100.06	99.896	0.3244	0.3248	0.0129
		2	30.10	100.33				
		3	29.89	99.63				
		4	29.86	99.53				
		5	29.98	99.93				

* Mean of five observations

TABLE 10

RECOVERY ANALYSIS OF FORMULATION
(LAFAXID-D) SIMULTANEOUS ESTIMATION METHOD

Drug	S. No	Amount present* (µg/ ml)	Amount added* (µg/ ml)	Amount estimated* (µg/ ml)	Amount recovered* (µg/ ml)	% Recovery*	Mean %	S.D.	% R.S.D.	S.E.
LAF	1	6.0155	9.6095	15.4880	9.4825	98.67	98.37	0.2761	0.2807	0.0306
	2	6.0155	12.046	17.7495	11.8440	98.32				
	3	6.0155	14.406	20.1382	14.136	98.125				
DOM	1	18.0659	28.8315	46.7167	28.7353	99.66	99.55	0.2023	0.2032	0.0224
	2	18.0659	36.0233	53.7822	35.7818	99.32				
	3	18.0659	43.2745	61.1326	43.1369	99.68				

* Mean of Three Observations

TABLE 11

OPTICAL CHARACTERISTICS OF LAFUTIDINE

AREA UNDER CURVE METHOD

Parameters	**At 276-271 nm**	**At 291-282 nm**
Beers law limit (μg mL^{-1})	4 – 20	4 – 20
Sandell's sensitivity (μg/cm^2/0.001 A.U)	0.020165	0.033825
Molar absorptivity	21229.38	12563.14
Correlation coefficient (r)	0.99976	0.99955
Régression equation (y = mx+c)	y = 0.049589x - 0.00395	y = 0.029564x - 0.00452
Slope (m)	0.049589	0.029564
Intercept (c)	- 0.00395	-0.00452
LOD (μg mL^{-1})	0.076977	0.128102
LOQ (μg mL^{-1})	0.23326	0.38818
Standard Error	0.00891	0.00742

TABLE 12

OPTICAL CHARACTERISTICS OF DOMPERIDONE

AREA UNDER CURVE METHOD

PARAMETERS	**AT 276-271 nm**	**AT 291-282 nm**
Beers law limit (μg mL^{-1})	12 – 60	12 – 60
Sandell's sensitivity (μg/cm^2/0.001 A.U)	0.01257	0.003938
Molar absorptivity	34255.231	109225.025
Correlation coefficient (r)	0.99968	0.9997
Régression equation (y = m*x*+c)	y = 0.0794x +0.008967	y = 0.25485x +0.01902
Slope (m)	0.07943	0.25485
Intercept (c)	0.008967	0.01902
LOD (μg mL^{-1})	0.012033	0.107255
LOQ (μg mL^{-1})	0.036463	0.325016
Standard Error	0.05071	0.16258

TABLE 13

QUANTIFICATION OF FORMULATION (LAFAXID-D)

AREA UNDER CURVE METHOD

Drug	Sample No.	Labeled amount (mg/ tab)	Amount found (mg/ tab)*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
LAF	1	10	9.932	99.32	98.606	0.8699	0.8822	0.0348
	2	10	9.832	98.32				
	3	10	9.762	97.62				
	4	10	9.858	98.58				
	5	10	9.949	99.49				
DOM	1	30	30.01	100.03	99.876	0.4135	0.41407	0.0165
	2	30	30.02	100.06				
	3	30	29.79	99.3				
	4	30	29.89	99.63				
	5	30	30.108	100.36				

* Mean of five observations

TABLE 14

INTRADAY AND INTERDAY ANALYSIS OF FORMULATION (LAFAXID-D) AREA UNDER CURVE METHOD

Drug	Sample No.	Labeled amount (mg/ tab)	Percentage obtained*		S.D.		% R.S.D.		Standard error	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
LAF	1	10	99.32	98.12	0.8590	0.7031	0.8658	0.7124	0.0954	0.0781
	2	10	100.03	99.48						
	3	10	98.32	98.49						
Mean			99.22	98.696						
DOM	1	30	99.56	99.6	0.2451	0.6621	0.2456	0.6629	0.0272	0.0735
	2	30	100.05	99.41						
	3	30	99.79	100.64						
Mean			99.8	99.88						

* Mean of five observations

TABLE 15

RUGGEDNESS STUDY (LAFAXID- D) AREA UNDER CURVE METHOD

Drug	Condition	Sample No.	Amount found (mg/ tab)*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
LAF	Analyst-1	1	9.932	99.32	99.018	0.4767	0.4815	0.01907
		2	9.896	98.96				
		3	9.958	99.58				
		4	9.832	98.32				
		5	9.891	98.91				
DOM	Analyst-1	1	30.06	100.2	99.976	0.3056	0.3057	0.01222
		2	29.98	99.95				
		3	29.89	99.63				
		4	30.108	100.36				
		5	29.92	99.74				

* Mean of five observations

TABLE 16

RUGGEDNESS STUDY (LAFAXID-D) AREA UNDER CURVE METHOD

Drug	Condition	Sample No.	Amount found (mg/ tab)*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
LAF	Analyst-2	1	9.732	97.32	98.356	0.818	0.8316	0.0327
		2	9.865	98.65				
		3	9.768	97.68				
		4	9.89	98.9				
		5	9.923	99.23				
DOM	Analyst-2	1	29.983	99.94	99.196	1.1036	1.1126	0.0441
		2	29.862	99.54				
		3	29.732	99.1				
		4	29.2	97.34				
		5	30.02	100.06				

* Mean of five observations

TABLE 17

RUGGEDNESS STUDY (LAFAXID-D) AREA UNDER CURVE METHOD

Drug	Condition	Sample No.	Amount found (mg/ tab)*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
LAF	Instrument-1	1	9.89	99.89	99.148	1.015	1.023	0.0406
		2	9.966	99.66				
		3	9.754	97.54				
		4	9.99	99.9				
		5	9.875	98.75				
DOM	Instrument-1	1	30.03	100.1	99.77	0.3674	0.3682	0.0146
		2	30.05	100.16				
		3	29.86	99.53				
		4	29.94	99.8				
		5	29.79	99.3				

* Mean of five observations

TABLE 18

RUGGEDNESS STUDY (LAFAXID-D) AREA UNDER CURVE METHOD

Drug	Condition	Sample No.	Amount found (mg/ tab)*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
LAF	Instrument-2	1	9.826	98.26	98.708	0.5337	0.5407	0.0213
		2	9.93	99.3				
		3	9.892	98.92				
		4	9.902	99.02				
		5	9.804	98.04				
DOM	Instrument-2	1	29.932	99.77	99.622	0.701	0.7037	0.028
		2	29.621	98.73				
		3	30.03	100.1				
		4	29.727	99.09				
		5	30.126	100.42				

* Mean of five observations

TABLE 19

RECOVERY ANALYSIS OF

(LAFAXID-D) AREA UNDER CURVE METHOD

Drug	S. No	Amout present* (µg/ ml)	Amont added* (µg/ml)	Amount estimated* (µg/ ml)	Amount recovered* (µg/ ml)	% Recovery*	Mean %	S.D.	% R.S.D.	S.E.
LAF	1	6.0155	9.6095	15.548	9.5325	99.19	98.573	0.5462	0.5541	0.0606
	2	6.0155	12.046	17.8395	11.824	98.15				
	3	6.0155	14.406	20.1882	14.1727	98.38				
DOM	1	18.0659	28.8315	46.9024	28.8365	100.01	99.9	0.1652	0.1653	0.0407
	2	18.0659	36.0233	53.9876	35.9217	99.71				
	3	18.0659	43.2745	61.3342	43.2683	99.98				

* Mean of Three Observations

TABLE 20

SYSTEM SUITABILITY PARAMETERS FOR THE OPTIMIZED CHROMATOGRAM RP-HPLC

PARAMETERS	LAFUTIDINE	DOMPERIDONE
Retention time	1.287	3.342
Tailing factor	0.5	1
Asymmetrical factor	1.30	1.69
Theoretical plates	1084	1513
Capacity factor	0.832	3.77
Resolution	Between LAF and DOM 7.26	

TABLE 21

OPTICAL CHARACTERISTICS OF LAFUTIDINE AND DOMPERIDONE RP-HPLC

PARAMETERS	LAFUTIDINE	DOMPERIDONE
λ_{max} (nm)	220	220
Beers law limit (µg/ml)	35-65	105-195
Correlation coefficient (r)	0.9999	0.9999
Regression equation (y = m*x* + c)	y = 18744.4214x + 8430.0714	y = 13596.2357x – 73555.7857
Slope (m)	18744.4214	13596.2357
Intercept (c)	8430.0714	-73555.7857
LOD (µg/ ml)	0.011058	0.015419
LOQ (µg/ ml)	0.0335102	0.046726
Standard Error	2147.368	14279.602

TABLE 22

QUANTIFICATION OF TABLET FORMULATION (LAFAXID- D) RP – HPLC

Drug	Sample No.	Labeled amount (mg/ tab)	Amount found (mg/ tab)	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
LAF	Low level (80 %)	10	10.04	100.42	99.68	0.7071	0.7093	0.0785
	Middlelevel (100 %)	10	9.96	99.62				
	High level (120 %)	10	9.9	99.01				
DOM	Low level (80 %)	30	29.81	99.37	99.39	0.2675	0.2673	0.0295
	Middlelevel (100 %)	30	29.74	99.14				
	High level (120 %)	30	29.9	99.67				

* Mean of three observations

TABLE 23

RECOVERY ANALYSIS OF FORMULATION (LAFAXID- D) RP-HPLC

Drug	S. No	Amount present (µg/ ml)	Amount added* (µg/ ml)	Amount found* (µg/ ml)	Amount recovered* (µg/ ml)	% Recovery*	Mean %	S.D.	% R.S.D.	S.E.
LAF	1 2 3	50 50 50	5 10 15	54.987 60.103 64.878	4.987 10.103 14.878	99.74 101.03 99.18	99.98	0.9487	0.9488	0.1054
DOM	1 2 3	150 150 150	15 30 40	164.756 179.912 190.078	14.756 29.912 40.078	98.37 99.70 100.195	99.42	0.9438	0.9492	0.1048

* Mean of Three Observations

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